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The Variability of Sugar Beet Constituents as Influenced by Year, Location, Variety, and Nitrogen Fertilization[1]

DeVere R. McAllister, Rex L. Hurst, Donald G. Woolley, Harold M. Nielsen, L. Elmer Olson, Delbert A. Greenwood, Homer M. LeBaron, and William H. Bennett[2]

Received for publication September 21, 1960

Introduction

The wide variability found in sugar beet constituents concerns farmers, processors, and researchers. An understanding of this variability and what causes it is essential to the continued profitable production of sugar beets and the extraction of the maximum amount of sugar. The reasons for this variability are only partially understood as the majority of investigators have considered single factors which can only give partial explanations. The purpose of this study was to determine the effect of soil, fertilization, climate, and variety upon the following constituents: marc, sucrose, total nitrogen, amino nitrogen, total glutamate, total anionic constituents, moisture, malic acid, oxalic acid, raffinose, and galactinol. Beet weight and beet girth measurements were also obtained and reported.

Review of Literature

The economic value of sucrose has focused attention on the amount of sugar in beets. McGinnis (12) sampled 50 consecutively grown beets from one row and found sugar content varying from 4.4 to 16%. The sugar content of 48 samples of seven beets each varied from 12.1 to 17.3%.

Owens et al. (13) found the marc content of 15 varieties of beets grown under the same conditions varied from 3 to 6%. He found a positive correlation between marc, araban, and sucrose contents in inbred beets. Araban in the marc varied so much that it may be subject to control by plant breeding.

[1] Contribution from the Department of Agronomy, Applied Statistics, and Chemistry, Utah Agr. Exp. Sta., Logan, Utah. Approved as Journal Paper No. 131 Utah Agr. Exp. Sta., Feb. 26, 1960. This work was conducted under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act of 1946. The contract was supervised by the Western Utilization Research and Development Division of the Agricultural Research Service. The authors appreciate the assistance given to this project by Perc A. Reeve, Farmers and Manufacturers Beet Sugar Association, Saginaw, Michigan; the University of California Agronomy Department, Davis, California; and W. F. Speth, College Ward, Utah. They also wish to acknowledge the assistance of Ruel Lamborn, Research Assistant, Department of Chemistry, Utah State University.

[2] Professor, Department of Agronomy; Professor and Head, Department of Applied Statistics; Graduate Research Assistant, Department of Agronomy (Currently Research Agronomist, Dominion Experiment Station, Lethbridge, Alberta, Canada); Assistant Professor, Department of Chemistry; Assistant Professor, Department of Chemistry; Professor of Biochemistry and Pharmacology, Department of Chemistry; Graduate Research Assistant, Department of Agronomy (Currently Research Agronomist, Virginia Polytechnic Institute); and Dean, College of Agriculture respectively, Utah State University.

Glutamic acid values usually have an inverse relation to percent sugar. Walker et al. (18) sampled beets from fields having different fertilizer levels and determined glutamic acid values ranging from 0.03 to 0.44%. Glutamic acid underwent a geometric increase with added nitrogen fertilizer. Hac et al. (9) reported the coefficient of variation for glutamic acid to be about twice as large as that for root weight and five times as large as that for sugar content.

The influence of nitrogen fertilizer upon percent sugar has been studied by many. Gardner and Robertson (6) reported a decrease in sugar percentage when nitrates were present in excess of the amount which could be used by the beets before harvest. Beet yields increased with increased nitrates but the percent sugar decreased until the maximum sugar yield was reached. Haddock (10) reported that sucrose and purity percentages were increased by heavy, frequent irrigations and a deficiency of available nitrogen. On the other hand, light irrigations and heavy nitrogen fertilization depressed sucrose and purity percentages. Haddock et al. (11) indicated that the glutamine and ammonia nitrogen components appeared to be most highly related to changes in sugar beet quality. They believed that glutamine nitrogen was of highest significance in quality variation. The concentration of nitrogen in this form was ten times that in the ammonia fraction.

Dahlberg (5) collected 24-hour composite cossette samples from ten suagr beet factories ranging in location from Kansas to Canada. Analyses of these samples gave the following results: ash content varied from 2.44 to 4.19%, nitrogen from 0.616 to 1.149%, P_2O_5 from 0.221 to 0.335%, and K_2O from 0.86 to 1.73%. From these analyses and that of hundreds of other beets, Dahlberg concluded that ripe beets of high sugar content and purity normally have the following characteristics: low ash, low total nitrogen, low nitrate, low sodium content and a CaO plus MgO content equal to 18% or more of the soluble ash.

Rounds et al. (14) attributed variations in raffinose content to varieties rather than to the effects of nitrogen fertilization. They obtained low correlation coefficients with raffinose vs. nonsugars. McGinnis (12) described raffinose as a nearly chemically unreactive sugar which seems to pass through all the processing operations and into the molasses. It was markedly more unreactive than sucrose or other di or tri saccharides.

Swink and Finkner (17) found an inverse relation between weight of beets and galactinol content.

Total anionic constituents, malic acid, and oxalic acid are not reported in recent articles on the constituents of sugar beets; however, these constituents were determined to see if they were related to or affected the sugar content of beets.

Materials and Methods

Experimental Plantings

North Logan, Utah — 1954: Sugar beets were planted April 15, on the Greenville Experimental Farm, North Logan, Cache County, Utah, and harvested October 20. This was a pilot planting made to develop procedures that would be used in the main study. Segmented seed of the variety US 22 Improved was seeded at a rate of seven pounds per acre and the resulting stand was thinned to an in-row spacing of 12 inches. Seven adjacent rows 24 feet long and 22 inches apart made up each plot. The Millville silt loam soil received 100 pounds P_2O_5 and 80 pounds N during the year. The plot area received 26 irrigations for a total of 31.7 inches of water plus 6.09 inches of natural precipitation during the growing season.

North Logan, Utah — 1955: Sugar beets were planted April 30, on the Greenville Experimental Farm, and harvested October 29. Regular seed of variety *A* (SP 53104-0) was used. A Planet Jr. hand seeder was used to sow 15 pounds of seed per acre. The beets were thinned by hand at the 4-leaf stage on June 8 to an in-row spacing of 12 inches. There were four plots each consisting of seven 60 foot rows 20 inches apart.

The soil, Millville silt loam, had a pH of 7.9, organic matter content of 2.4%, total soluble salts 0.03%, P_2O_5 59 pounds per acre, K_2O 130 pounds per acre, lime 44 to 50%, and a mechanical analysis of 26% sand, 58% silt, 16% fine clay, and 21% clay. Texture, pH, lime content were similar to a depth of 6 to 8 feet. Organic matter drops off rapidly with depth. Previous crops on this field were: 1950 — tomatoes which received 200 pounds of treble superphosphate (46% P_2O_5) per acre, 1951 — grain, 1952 — alfalfa and barley, 1953 — alfalfa, and 1954 — potatoes.

All plots received 100 pounds of available P_2O_5 per acre, broadcast on April 20, 1955, half of the plots were side-dressed on May 2 with 80 pounds of N (ammonium sulfate) per acre and with 125 pounds of N. The latter half received an additional 125 pounds of N per acre side-dressed on July 22, 1955.

The beets were irrigated nine times receiving 16 inches of water plus 6.72 inches of natural precipitation.

North Logan, Utah — 1956: Beets were planted May 1, on the Greenville Experimental Farm, and harvested November 2 and 3. Variety *B* was planted at a per acre rate of 15 pounds. The plots were thinned to an in-row spacing of 12 inches on June 8 and 9. There were 12 plots each 11.7 feet × 35 feet. Each plot consisted of 7 rows spaced 20 inches apart and 35 feet long.

The soil, Millville silt loam, had a pH of 8.0, total soluble salts less than 0.02%, and available P_2O_5 55 pounds per acre. Lime and available K_2O were not determined as previous analyses had shown these to be plentiful. Previous crops on this field were: 1950 — canning peas which received 100 pounds P_2O_5 followed by alfalfa, 1951 — alfalfa (2 crops), 1952 — alfalfa (2 crops), 1953 — potatoes which received 100 pounds P_2O_5.

All plots received 200 pounds per acre of treble superphosphate (45% P_2O_5) broadcast and harrowed in before planting. Four plots received no additional N, four received 80 pounds per acre of N (ammonium sulfate) May 19 side-dressed 3 inches from the beets and 3-inches deep. The remaining four plots received in like manner 125 pounds of N per acre on May 19 and an additional 125 pounds per acre of N on July 22.

Eight irrigations of from 2 to 3 inches of water each were given. Natural precipitation amounted to 4.71 inches.

College Ward, Utah — 1955: Beets were planted May 20, on the Willy Speth farm, College Ward, Cache County, Utah, and harvested on October 15. A hand drill was used to plant 16 pounds of seed per acre of varieties *A* and *B*. Seedling emergence began May 23 and beets were hand thinned at the 4-leaf stage on June 15 to an in-row spacing of 12 inches. There were four plots each consisting of seven 60 foot rows 20 inches apart.

The soil was classified as Trenton silty clay and had a pH of 8.1, organic matter 4.2%, total soluble sales 0.09%, P_2O_5 76 pounds per acre, K_2O 800 pounds per acre, and a mechanical analysis of 8% sand, 43% silt, 49% fine clay, and 69% clay. Previous crops on this field were: 1951 — winter wheat, 1952 — winter wheat, 1953 — winter wheat, 1954 — corn which received 20 tons of manure per acre.

All plots received 100 pounds of available P_2O_5 per acre broadcast before seeding. Half of the plots received 80 pounds of N and the other half received 125 pounds per acre at seeding time and an additional 125 pounds of N per acre side-dressed on July 25.

The beets were irrigated five times receiving approximately 4 inches of water per application. Precipitation data from Utah

State University, the nearest station (5 miles away), showed that 6.46 inches of rain fell.

Davis, California — 1955: Beets were planted March 17, on the Campbell Tract, California Experiment Station, Davis, California, and harvested September 28. A commercial drill was used to seed variety *A* only. Due to dry soil a light irrigation was given on March 19 and excellent emergence resulted. The beets were hand thinned to an in-row spacing of 12 inches on April 28 at the 4-leaf stage. There were four plots with each plot consisting of 4 beds, 2 rows per bed, 60 feet long with a 25 × 14-inch row spacing.

The soil, Yolo loam, had a pH of 7.5, organic matter 1.3%, total soluble salts 0.04%, P_2O_5 37 pounds per acre, K_2O 270 pounds per acre, and a mechanical analysis of 40% sand, 44% silt, 16% fine clay, and 23% clay. Previous crops on this field were: 1951 — sugar beets, 1952 — dryland barley, 1953 — dryland barley, and 1954 — field beans.

All plots received 100 pounds of N per acre (ammonium nitrate) side-dressed 2-inches deep and 6 inches from the row on May 12 and 80 pounds of N side-dressed 2-inches deep and midway between beds on June 30.

Ten irrigations were applied with the soil wetted to a depth of 3 to 4 feet each time and at no time did the beets wilt. Natural precipitation recorded at Davis totaled 4.12 inches of which 2.16 inches fell in April.

Saginaw, Michigan — 1955: Beets (Variety A) were planted April 16, two miles west of Saginaw, Michigan, and harvested October 24. They were blocked and thinned on May 27 with a 90% stand reported on June 6. The plot consisted of seven rows, spaced 28 inches apart and 60 feet long.

The soil, Kawkawlin loam, had a pH of 7.2, organic matter 4.6%, total soluble salts 0.04%, CO_2 soluble PO_4 1 ppm, and CO_2 soluble NO_3 3 ppm. Previous crops on this field were: 1952 — white beans, 1953 — wheat, and 1954 — white beans. One-hundred fifty pounds of 3-12-12 fertilizer per acre had been applied each of these three years.

The plot received 500 pounds of 6-24-12 fertilizer per acre applied 2 inches below the seed at planting time. On June 4 the beets were side-dressed with 40 pounds of liquid nitrogen per acre.

These beets were grown without irrigation but 20.56 inches of rain fell during the season.

Harvesting and Processing Procedures

1954: Fifty beets were sampled at random from the center three rows of the seven-row plot and refrigerated at 33° F. Three days later the refrigerated beets were washed with cold water, numbered, weighed, and refrigerated at 33° F. During November the beets were rasped and aliquots frozen for chemical analyses.

1955: Fifty beets were sampled from the center three rows (four rows in California) of each plot as follows: a 5-foot length was discarded from each end of each plot; the remainder of the plot was divided into five equal sections; and a 10 beet sample, made up of five random pairs of beets, was taken from each section. In order for a beet to have been included in the sample, it must have had an adjacent in-the-row mate and both must have been free from adjacent skips and/or multiple beets. The beets were lifted with a garden fork, labeled, topped, and placed in an ice water bath.

All beets were topped by removing the petioles by hand and rubbing the crowns with an ordinary plastic household scouring pad. This left the crown on each beet but removed most of the green material as well as the scar tissue. As soon as the beets were topped they were placed in ice water (in 55 gallon drums) and held at 0° C while they were transported to the laboratory.

The beets were cleansed with cold water, weighed to the nearest 1/10 pound, and classified into one of four shape categories. The maximum girth of each beet was determined. The whole beets were individually chopped in a Hobart Food cutter (Model T 215) as the bowl rotated for approximately 2 minutes. Twelve 10-gram samples of the macerated material were weighed out from each beet, wrapped in aluminum foil, and frozen immediately. The samples were held at –5° F to await chemical analysis. The interval between digging and completion of processing was approximately 3 hours.

1956: Instead of selecting five random pairs of beets from each section as in 1955, 10 beets were selected by complete randomization within each section. Fifty beets were thus harvested from each plot.

Chemical Methods and Procedures

Sample Preparation: The frozen, aluminum-foil wrapped samples of chopped beets were weighed on an analytical balance, the frozen sample removed to a 250 ml beaker and the foil weighed to get the net weight of the sample. As many individual determinations as possible were made on each individual sample.

Duplicate determinations were made on two or more separate samples of the same beet.

The extraction procedure was a modification of that used by Goodban, Stark, and Owens (7). Fifty ml of boiling distilled water were added to the beaker which was then placed on a variable temperature hot plate and maintained between 78° and 82° C for 30 minutes. Each beaker contained a thermometer and was covered with a watch glass. At the end of the 30 minute period the water was carefully decanted into a 100 ml volumetric flash, another 50 ml portion of hot distilled water was added to the pulp, washing down any material adhering to the side of the beaker, and heating was repeated for another 30 minutes. This portion was decanted into the volumetric flask at the end of the time interval. Small portions of hot water were added to the pulp and decanted into the volumetric flask until the liquid reached the mark. Usually the evaporation loss amounted to 10 to 20 ml. On cooling to room temperature the volume was finally adjusted to 100 ml using distilled water.

Chemical Procedures: The salts of oxalic and malic acid in the sugar beet extract were converted to acids by passing some of the extract over a Dowex 50 hydrogen cation exchange resin and the acids were then absorbed on a Dowex 1 anion exchange resin. The acids were then fractionally eluted with 30 ml of 0.35 N ammonium carbonate. Lactic and glycolic acids were eluted first and discarded. Malic acid was determined by a slight modification of the method of Goodban and Stark[3] as outlined by Stark, Goodban and Owens (16) page 564.

Oxalic acid was then eluted from the resin with 50 ml of 0.55 N ammonium carbonate. The eluate was made acid with 2.5 ml of concentrated sulfuric acid, 1 ml of 1% manganese sulfate was added, and the solution titrated with 0.005-0.01 N potassium permanganate. The end point was reached when 0.15 ml of the potassium permanganate produced a color which persisted for at least 10 seconds. Citric acid, which was present, was oxidized very slowly by the permanganate at the temperature of the titration.

Sucrose was determined by the method found in the Association of Official Agricultural Chemists (2) pages 347, 348 and 507.

Total nitrogen was determined by the procedure found in the Association of Official Agricultural Chemists (1) page 13.

[3] Private communication from Dr. H. S. Owens, deceased, formerly supervisor, Sugar Beet Unit, Field Crop Utilization Section, Western Utilization Research Branch, U. S. Department of Agriculture.

Amino nitrogen was isolated on Dowex 50 Cation exchange resin and estimated by the ninhydrin method as modified by Cocking and Yemm (4).

Total glutamate was determined by the microbiological method of Hac, Walker, and Dowling (9) and Hac, Long and Blish (8).

Total anionic constituents were determined by the conversion of salts to acid form on Dowex 50 cation exchange resin followed by titration with sodium hydroxide.

In determining marc the ground samples were extracted with 80% alcohol, dried, and weighed.

In determining raffinose and galactinol, the frozen samples were partially thawed, then pressed at 9,000 pounds per square inch in a Carver laboratory press (Serro and Brown, 15). The expressed juice was used directly or diluted with water to bring the sample within range of the standards.

A special drying-developing oven was constructed essentially according to plans supplied by Robert J. Brown of the Great Western Sugar Company, Denver, Colorado, (Drawing No. 9419).

The minor modifications of the basic methods were these: raffinose: (a) Standards were made up to give 4, 8, 12, and 16 micrograms in a 10 microliter spot. It was necessary to use a sucrose base for these standard solutions. (b) An n-propyl alcohol-ethyl acetate-water was used as a solvent mixture for about 22 hours. (c) the α napthol solution was applied to the paper by drawing the paper quickly through the solution in a tray and immediately hanging it up to dry. This gave very even distribution without streaking the raffinose spots. Galactinol: (a) Standard spots contained 2, 4, 8, and 12 micrograms. The galactinol used as standard was furnished by Mr. Robert J. Brown of the Great Western Sugar Company.

Results and Discussion

Chemical analyses of the beets produced in 1954 are summarized in Table 1. The high variability due to determinations was believed to be due to inherent limitatons of the analytical methods, limited experience of the analysts, long sample storage period during which temperature controls were not adequate, inherent variability of the sugar beets, and the low concentration of some of the constituants in the beets. Correlation coefficients between sucrose and total nitrogen and between sucrose and total anionic constituents were nonsignificant.

It should be remembered that this was a preliminary trial used to develop procedures to be used in the major trials of 1955 and 1956.

Table 1.—The mean, and coefficient of variation values for the constituents of the 50 sugar beets sampled in Utah in 1954.

Constituent		Mean	Coefficient of variation	
			Single beet[1]	Single determinations[2]
Glutamate	% fresh weight	0.1240	75.4	23.8
Reducing sugar	% fresh weight	0.17	48.2	15.2
Amino nitrogen	% fresh weight	0.0184	44.6	14.1
Anionic constituents	meq/100g of sucrose	29.16	22.2	7.0
Total nitrogen	% fresh weight	0.1860	18.3	5.8
Total marc	% fresh weight	5.62	13.3	4.2
Dry matter	% fresh weight	25.44	7.4	
Sucrose	% fresh weight	17.92	6.6	2.1

[1] The standard error of a single beet, eliminating determination error and assuming randomized sampling, as a percent of the mean. Coefficient of variation of single beets.

[2] The standard error of a single determination expressed as percent of the mean. Coefficient of variation of single determinations.

The results of the analyses of the beets produced in 1955 and 1956 are summarized in Table 2. Mean comparisons can be made with the 1954 data but it is difficult to draw conclusions because the constituents reported and the units of measurement differed. Direct comparisons may be made between coefficients of variation. Glutamate was the most variable and sucrose the least variable constituent in both years.

Other constituents differ in rank but were essentially in the same order. The 1956 results represent 50 beets of one variety sampled at one location (Utah) and grown under one nitrogen level. The results for 1955 (Table 2) repreesnt 11 lots of 50 beets each which include 2 varieties, 3 locations (Utah, California and Michigan) and 4 nitrogen levels.

Correlations: Simple correlation coefficients involving all possible combinations (66 in all) were calculated for each nitrogen level and location (11 in all) in 1955 and 1956. The results are summarized and presented in Table 3. Sucrose was negatively correlated with all chemical constituents except galactinol and was positively correlated with marc. No correlation was found between sucrose $\times$ beet weight, sucrose $\times$ beet girth, or sucrose $\times$ galactinol. Other significant correlations may be seen in Table 3.

The negative correlation between sucrose $\times$ glutamate is in agreement with Walker et al. (18). The negative correlations between sucrose $\times$ total nitrogen and sucrose $\times$ amino nitrogen agrees with Haddock (10).

Table 2.—Mean and coeffcients of variation for sugar beets sampled in Utah, California, and Michigan in 1955 and in Utah in 1956.

Constituent		Mean 1955	Mean 1956	Coefficients of Variation 1955		1956	
				Single beet[1]	Single determinations[2]	Single beet[1]	Single determinations[2]
Glutamate	mg/100g beet	58.7	59.5	64.1	20.2	48.2	15.2
Galactinol	% fresh weight	.057	.025	51.0	16.1	36.4	11.5
Amino nitrogen	mg/100g beet	37.9	40.0	41.9	13.2	43.2	13.7
Beet weight	pounds per beet	2.22	2.19	41.8		33.0	
Raffinose	% fresh weight	.112	.172	29.2	9.2	30.4	9.6
Malic acid	mg/100g beet	41.2	29.7	27.7	8.8	29.3	9.3
Beet girth	inches per beet	12.6	11.6	16.0		12.8	
Total nitrogen	mg/100g beet	227.5	216.2	15.9	5.0	19.9	6.3
Oxalic acid	mg/100g beet	109.3	96.6	15.5	4.9	16.0	5.1
Anionic constitutents	meq/100g beet	8.87	7.90	15.3	4.8	15.1	4.8
Marc	% fresh weight	4.93	4.90	9.5	3.0	10.9	3.4
Sucrose	% fresh weight	14.9	15.0	7.1	2.2	8.3	2.6

[1] The standard error of a single beet, eliminating determination error and assuming randomized sampling, as a percent of the mean. Coefficient of variation of single beets.

[2] The standard error of a seingl determination expressed as percent of the mean. Coefficient of variation of single determinations.

Table 3.—Tabulation of the significance of the respective correlation coefficients for the 11 different lots of beets.

	Comparison	Number of times (out of 11) correlation coefficients were significant			
		.05 level		.01 level	
		Positive	Negative	Positive	Negative
1	weight × girth	11		11	
2	sucrose				
3	marc				
4	malic acid	7		3	
5	oxalic acid	1			
6	anionic constituents	2			
7	total nitrogen				
8	amino nitrogen				
9	glutamate	1			
10	galactinol				
11	raffinose	1			
12	girth × sucrose				
13	marc		1		
14	malic acid	7		3	
15	oxalic acid				
16	anionic constituents	6			
17	total nitrogen				
18	amino nitrogen				
19	glutamate	1			
20	galactinol		1		
21	raffinose				
22	sucrose × marc	10		5	
23	malic acid		8		8
24	oxalic acid		9		7
25	anionic constituents		10		8
26	total nitrogen		3		1
27	amino nitrogen		6		4
28	glutamate		5		4
29	galactinol				
30	raffinose		3		1
31	marc × malic acid		8		5
32	oxalic acid	1	1		
33	anionic constituents		2		
34	total nitrogen	3			
35	amino nitrogen		2		
36	glutamate		3		1
37	galactinol	1			
38	raffinose		1		
39	malic acid × oxalic acid	6		2	
40	anionic constituents	9		8	
41	total nitrogen				
42	amino nitrogen	4			
43	glutamate	5			
44	galactinol	1		1	
45	raffinose				

TABLE 3.—Continued.

		Number of times (out of 11) correlation coefficients were significaant			
		.05 level		.01 level	
	Comparison	Positive	Negative	Positive	Negative
46	oxalic acid × anionic constituents	10		8	
47	total nitrogen	1		1	
48	amino nitrogen	3		1	
49	glutamate	4		1	
50	galactinol	1		1	
51	raffinose	1		1	
52	anionic const. × total nitrogen	7		5	
53	amino nitrogen	6		4	
54	glutamate	5		3	
55	galactinol	1			
56	raffinose	1			
57	total nitrogen × amino nitrogen	10		10	
58	glutamate	10		8	
59	galactinol		2		
60	raffinose				
61	Amino nitrogen × glutamate	10		9	
62	galactinol		3		
63	raffinose				
64	glutamate × galactinol	2	3		2
65	raffinose	1		1	
66	galactinol × raffinose		1		

Constituents and Coefficients of variation: Table 4 gives the mean constituent value for each variety, year, location, and rate of nitrogen fertilization. The corresponding coefficient of variation for a single determination is included. Each figure is based on a 50 beet sample and duplicate determinations, except for beet weight and girth on which only single measurements were made.

The coefficient of variation for glutamate is about 1.5 times that for beet weight and 8.8 times that for sucrose. These agree essentially with Hac et al. (9) who reported multiples of 2 and 5, respectively.

Equating the coefficient of variation for sucrose equal to one and relating the other coefficients to it gives the following: (1955)

Sucrose	1	malic acid	3.8
marc	1.3	raffinose	4.0
anionic constituents	2.1	beet weight	5.7
oxalic acid	2.1	amino nitrogen	5.7
total nitrogen	2.2	galactinol	7.0
beet girth	2.2	glutamate	8.8

Table 4.—Constituents of sugar beets and coefficients of variation in relation to year, location, variety, and rate of nitrogen fertilization.

Year	Location	Variety	Applied N per acre	Glutamate Mean mg/100g beet	C.V.[1]	Galactinol Mean % fresh weight	C.V.[1]	Amino nitrogen Mean mg/100g beet	C.V.[1]	Beet weight Mean pounds per beet	C.V.[1]
			lb								
1955	Davis, California	A	180	165.6	18.5	.036	13.1	46.2	11.1	3.02	12.5
	Saginaw, Michigan	A	70	85.8	10.9	.040	10.6	59.0	9.9	2.51	11.7
	College Ward, Utah	A	80	24.5	13.4	.082	18.3	25.9	15.1	2.20	11.6
		A	250	24.4	14.4	.057	9.6	27.7	14.8	2.25	13.1
		B	80	32.2	13.0	.056	18.1	31.1	12.7	2.53	13.0
		B	250	35.8	13.2	.056	11.4	25.5	16.4	2.13	15.4
	North Logan, Utah	A	80	49.9	13.6	.059	18.8	34.5	13.2	2.01	11.9
		A	250	84.1	17.6	.076	11.7	48.9	10.5	2.05	13.2
		B	80	29.0	20.3	.057	21.7	25.0	19.3	1.75	13.2
		B	250	64.3	9.4	.051	11.2	55.7	13.5	1.79	16.1
1956	North Logan, Utah	B	80	59.5	15.2	.025	11.5	40.0	13.7	2.19	10.3

Year	Location	Variety	Applied N per acre	Raffinose Mean % fresh weight	C.V.[1]	Malic acid Mean mg/100g beet	C.V.[1]	Beet girth Mean inches per beet	C.V.[1]	Total nitrogen Mean mg/100g beet	C.V.[1]
			lb								
1955	Davis, California	A	180	.044	7.0	39.1	10.1	13.5	5.0	184.1	6.2
	Saginaw, Michigan	A	70	.044	9.3	30.5	9.6	13.6	4.9	289.0	4.0
	College Ward, Utah	A	80	.116	9.6	49.8	8.2	12.6	4.7	185.9	3.8
		A	250	.129	12.1	48.4	10.8	12.6	5.4	205.7	4.1
		B	80	.126	8.8	48.3	9.0	13.4	4.7	208.1	4.5
		B	250	.109	6.4	46.4	7.9	12.6	5.1	204.8	5.0
	North Logan, Utah	A	80	.136	8.0	32.3	6.0	12.1	5.2	234.3	5.4
		A	250	.131	9.1	48.7	7.4	12.7	5.1	280.5	3.1
		B	80	.143	6.8	29.9	8.5	11.3	4.9	188.3	9.4
		B	250	.146	8.6	39.0	7.4	11.5	5.4	294.7	4.7
1956	North Logan, Utah	B	80	.172	9.6	29.7	9.3	11.6	4.1	216.2	6.3

[1] Coefficient of variation of a single beet assuming no determination error and completly randomized sampling.

Table 4.—Constituents of sugar beets and coefficients of variation in relation to year, location, variety, and rate of nitrogen fertilization.—Cont.

				Oxalic acid		Anionic constituents		Marc		Sucrose	
Year	Location	Variety	Applied N per acre	Mean mg/100g beet	C.V.[1]	Mean meq/100g. beet	C.V.[1]	Mean % fresh weight	C.V.[1]	Mean % fresh weight	C.V.[1]
			lb								
1955	Davis, California	A	180	121.5	5.3	8.6	5.1	4.2	3.4	13.5	2.7
	Saginaw, Michigan	A	70	101.2	4.8	8.9	4.2	4.6	3.2	14.5	2.8
	College Ward, Utah	A	80	121.0	4.8	9.8	4.5	4.8	3.1	14.6	2.1
		A	250	122.3	5.0	10.4	5.2	4.9	2.8	14.1	2.9
		B	80	120.6	4.4	9.8	4.3	4.9	2.6	15.0	1.6
		B	250	120.8	3.9	9.3	4.0	5.0	2.8	14.7	1.5
	North Logan, Utah	A	80	92.3	5.4	7.7	5.1	5.3	3.4	15.9	1.8
		A	250	118.0	3.8	10.0	4.3	5.2	2.7	15.0	2.0
		B	80	88.9	6.2	6.3	5.5	5.1	3.0	16.0	2.2
		B	250	98.7	5.1	7.9	5.6	5.3	3.1	15.9	2.4
1956	North Logan, Utah	B	80	96.6	5.1	7.9	4.8	4.9	3.4	15.0	2.6

[1] Coefficient of variation of a single beet assuming no determination error and completely randomized sampling.

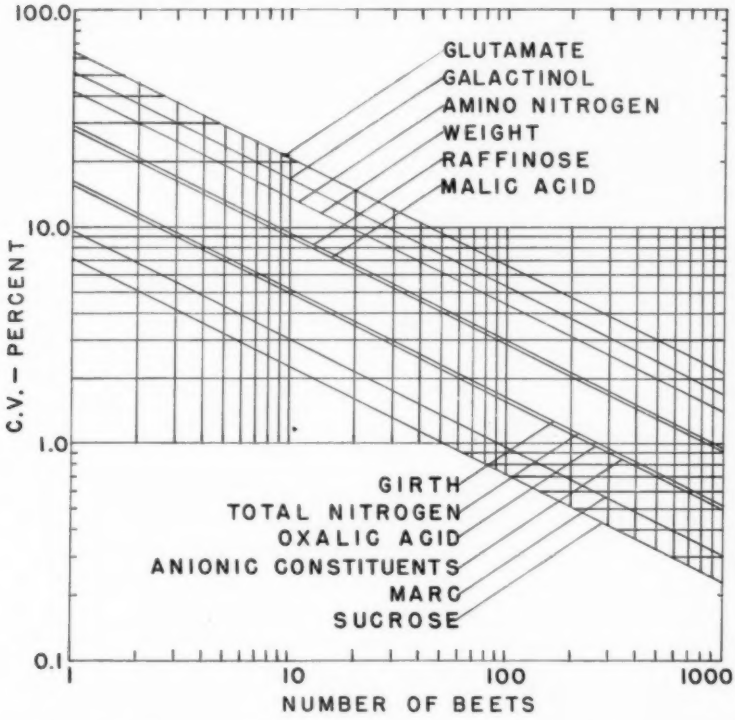

Figure 1.—Coefficient of variation of a mean assuming completely randomized sampling and the elimination of errors in determination as related to the number of beets sampled (Standard error of a mean as a percent of the mean).

Figure 1 graphically portrays coefficients of variation of the means assuming completely randomized sampling and the elimination of errors in determination as related to the number of beets in a composited sample. This figure may be used to predict the number of beets which must be sampled and composited before analysis if a particular coefficient is desired. For example to determine glutamate with a 10% coefficient of variation requires a composited 45 beet sample. On the other hand this sample, analysed for sucrose, would have a 1.2% coefficient of variation. Glutamate was about eight times as variable as sucrose. Other comparisons may be made.

Recommendations: Duplicate analyses are recommended for constituents having high determinational errors, e. g. glutamate, galactinol, amino nitrogen, raffinose and malic acid (Table 2). Drawing the line between duplicate and single analyses should be determined by the cost of running determinations versus the addition of more beets. When the determinational error is small more gain can be achieved by sampling additional beets. When the determinational error is large it may be necessary to run multiple determinations and in addition to sample more beets.

There was no significant gain or advantage achieved by spreading the sampling over the whole plot. The size of the plot was such that the first 50 beets were as good a sample as any 50 beets obtained by any elaborate procedure. The variability encountered in these uniform small plots was not environmental but genetic and only as such reacted with environment was it not uniform.

The variability was surprisingly uniform over all locations. The nitrogen fractions seemed to be related to nitrogen fertilization. Variations between locations were great; however, no attempts were made to draw these out. The variations measured and expressed were those found within plots.

Summary

Sugar beets were grown in Utah (1954, 1955, 1956), California (1955), and Michigan (1955) to study constituent variability as influenced by year, location, variety and nitrogen fertilization.

Twelve 50 beet samples were taken. The 1954 sample (50 beets) was selected at random for three adjacent rows 22 inches apart and 24 feet long. The ten 1955 samples were selected as five random sampled pairs of beets from each of five 10-foot long adjacent, end-to-end sections of a 3-row (4 row in California) by 50-foot plot area. The 1956 sample was selected at randoom 10 beets at a time from five 5-foot long, adjacent, end-to-end sections of a 3-row by 25-foot plot area.

Each beet was lifted, topped, labeled, and placed in ice water while transported to the laboratory. At the laboratory each beet was cleaned, weighed, measured, chopped, sampled, and frozen independently. About 3 hours elapsed between digging and sample freezing.

Laboratory analyses made on the basis of fresh weight included: mg/100g for glutamate, amino nitrogen, total nitrogen, malic acid and oxalic acid; meq/100 g for anionic constituents; and percent for galactinol, raffinose, marc, and sucrose. Duplicate determinations were made on each test for each constituent.

Statistical analyses of the data gave negative correlations between sucrose and the other chemical constituents except galactinol. Sucrose and marc were positively correlated but no relationship was evident between sucrose × beet weight and sucrose × beet girth. Sucrose was the only constituent which was consistently changed in the same direction (in this case downward) by an increase in nitrogen fertilization. The nitrogen constituents (glutamate, amino nitrogen and total nitrogen) were inconsistent in this respect.

Changes in coefficients of variation were graphically portrayed in relation to the number of beets in a composited sample. Using this graph one may predict the number of beets which must be sampled and composited before analysis if a particular coefficient of variation for any constituent is desired. Glutamate was the most variable and sucrose the least variable constituent measured. Varietal differences were not consistent and were usually not great.

Large determinational errors suggest a need for duplicate analyses and the addition of more beets whereas only single analysis may be needed where small determinational errors are encountered.

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Increased Yield and Purity of Hybrids from the Ovana Fodder Beet

MYRON STOUT, GEORGE K. RYSER, ALBERT M. MURPHY AND F. V. OWEN[1]

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Introduction

High yield of sugar per acre combined with high purity and a high degree of disease resistance is the ultimate goal of sugar beet breeders, farmers, and processors. These three factors may be equally important to the economy of a healthy industry, but usually a compromise is made in choosing the best variety to grow in a given area. Under epidemic disease conditions, disease resistance may be the controlling factor in obtaining high yields. In this case quality is most likely to suffer. Recent increases in the use of nitrogen fertilizers have also favored high yields of lower quality beets. For a long time sugar beets have been referred to as "yield types" or "sugar types" because these two characters do not usually occur together. However, recent progress in hybrid breeding has shown that these characters are not entirely incompatible. Controlled hybridization by use of genetic male sterility (4, 5)[2] offers many new possibilities for synthesizing desirable combinations of characters. A second-generation backcross from the Ovana fodder beet to sugar beets, designated SL 630, may be considered an example of such possibilities.

Normally the roots of sugar beets do not increase in size very rapidly until they develop a fairly large canopy of leaves. However, a rather wide heritable variation exists with regard to the time required to reach this stage of rapid root growth. Savitsky (9) has shown that yield types reached this development stage earlier than sugar types and that fodder beets reached this stage much earlier than either type of sugar beets. From June until September fodder-beet roots were more than twice the size of sugar beet roots. Obviously any decrease in the time required from planting to the stage of rapid root growth should be equivalent to an extension of the growing season and should increase yields accordingly. If heterosis depends upon synergy between dissimilar genes, hybrids between fodder beets and sugar beets might be expected to produce some valuable new material from which selections can be made for desirable characters.

[1] Physiologist, Research Agronomists and Geneticist, respectively, Agricultural Research Service, Crops Research Branch, U. S. Department of Agriculture.

[2] Numbers in parentheses refer to literature cited.

Breeding Materials and Methods

Included in the parentage of the hybrid SL 630 was the Ovana fodder beet, the sugar type variety US 35, and the high-sugar high-purity inbred CT8. Seed of the Ovana fodder beet was obtained from Dr. K. J. Frandsen of the Otoftegaard Breeding Station, Taastrup, Denmark. In common with other vigorous-growing fodder beet varieties the Ovana has a prodigious growth potential but is low in sugar and purity. It is also highly susceptible to curly top. Unlike roots of many other European fodder beet varieties the Ovana roots are not highly colored with red or orange pigment. They are white like sugar beets but are characterized by green color on the upper part of the roots exposed to sunlight because of their protrusion above the soil surface.

Roots of the Ovana fodder beet variety have certain characteristics similar and dissimilar to those of sugar beets. They are similar to sugar beets in amino nitrogen, very much lower in respiration rate, much higher in sodium content, and slightly higher in potassium. However, the potassium content of Ovana roots is much lower than one might expect in sugar beets with a very low sugar and high sodium content.

US 35 is a sugar-type, curly-top-resistant variety developed by selection from US 22/3. After the discovery of Mendelian male sterility in US 35 a backcross population was developed in which 50 percent of the plants were *aa* recessive with aborted pollen. Mendelian male-sterile *aa* segregates from this backcross population were hybridized with Ovana pollinators for the production of the F_1 generation hybrid. The F_1 hybrid was used to pollinate other male-sterile US 35 plants for production of the b_1 generation hybrid (See Figure 1).

The inbred CT8 (6, 8) was derived from a single self-fertile beet selected in 1952. It is a high-sugar type, high-purity inbred moderately low in amino nitrogen, sodium, potassium, and respiration rate. Previous field tests with hybrids of CT8 have indicated only average to poor combining ability. Beets of the inbred CT8 were relatively high in curly-top resistance, but at Jerome, Idaho, they were readily dwarfed by the disease without showing typical leaf symptoms. Mendelian male sterility was observed in selfed progeny of the original CT8. This inbred can, therefore, be used as a male or female parent in hybrid combinations.

A single beet from the b_1 population US 35 $\times$ (US 35 $\times$ Ovana) was used as the female in producing the hybrid SL 630. Details of the selection were as follows: From a field planting

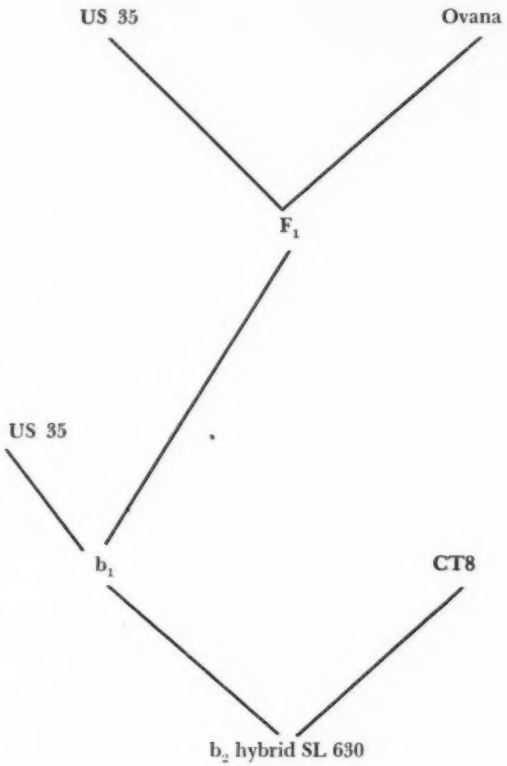

Figure 1.—Parentage of the b_2 generation hybrid sugar beet SL 630. The b_1 parentage consisted of one highly selected beet. (See Table 1)

of approximately 2,000 beets of the b_1 population, 158 individuals were selected for desirable root type. Some of the larger individuals resembling fodder beets as well as smaller ones more typical of the sugar beet parent were rejected. A preliminary test for dry substance was made with the Abbe refractometer (10) and the 15 highest were chosen for more complete chemical analysis. Sugar, total soluble solids, amino nitrogen, sodium, and potassium were determined. The highly superior beet No. 487 became the female parent of hybrid SL 630. The outstanding qualities of beet No. 487 in comparison with the average of the selected group of 15 from which it was chosen is shown in Table 1. Beet No. 487 was not only much larger but was superior in

Table 1.—Record of parental beet number 487 selected from the b_1 generation [US 35 × (US 35 aa × Ovana)] compared with the average of 15 selected sister beets from the same population grown at the same location.

Beet number	Root weight	Sugar	Purity	Amino N	Na	K
	grams	percent	percent	percent	ppm	ppm
487	2500	17.6	91.9	0.24	280	2210
Average of 15 selected beets	1771	17.0	88.4	0.46	290	3280

all the chemical characteristics related to quality that were measured. Small longitudinal sectors, including crown buds, were cut from each beet and these were grown in the greenhouse for observation of male sterility. When flowering occurred in March 1956, eight of the beets including No. 487 were found to be male-sterile segregates. The larger portion of each of the selected beets was thermally induced in the cold frame. These larger portions of the eight *aa* segregates were then planted in a garden isolation plot in April 1956, with the inbred CT8 as pollinator. Beet No. 487 produced 532 grams of hybrid seed which was labeled SL 630. Preliminary testing in 1957 showed that SL 630 was the most outstanding hybrid. However, one sister progeny, SL 631, was also very high in yield and quality. This fortunate selection in the b_1 generation and the preliminary progeny testing may be regarded as an important key to the discovery of the superior hybrid SL 630.

Methods of Evaluating Progenies

After preliminary field tests in 1957 had shown SL 630 and its sister hybrid SL 631 to be superior to other hybrids, SL 630 was extensively tested at Jerome, Idaho, and at Salt Lake City, Utah, in 1958. All parental lines and hybrids except the inbred CT8 were included in the test. The inbred CT8 was not included in the replicated test because of its poor vigor but it was included in adjacent unreplicated plots for general observation. The five varieties were replicated in randomized blocks. The data at Salt Lake City were based on twenty replications of 2-row plots 22 feet long; at Jerome, Idaho, eight replications of 2-row plots 50 feet long. The rows were 20 inches apart at Salt Lake City and 22 inches apart at Jerome, Idaho. The Jerome plots were planted on soil classified as Portneuf silt loam, deep phase, good drainage (1). The soil was low in salinity and high in fertility but not excessive in nitrogen. The plots at Salt Lake City were planted on Welby fine sandy loam (3) which was very high in salinity and fertility. Two spacings, 10 inches versus 15 inches between beets, were compared. An extra amount of

nitrate was added to half these plots. The varieties were randomized within each spacing and the spacings were alternated systematically within fertility levels. The extra-high fertility made practically no difference in yield or chemical analysis, and 15-inch spacing gave the same yield as the closer spacing with only a small reduction in quality; so the data for spacings and fertility levels were combined in Table 2. Spacings within the rows at Jerome was uniform. The beets were spaced approximately 12 inches apart.

Two 10-beet samples per plot were taken for sugar and other chemical analyses at both Jerome and Salt Lake City. Pulp samples from the Jerome plots were frozen and all chemical analyses were made at Salt Lake City. In addition to the usual sugar and apparent purity determinations, analyses were made for amino notrogen, sodium, and potassium by methods previously described (11). Amino nitrogen as glutamine reported here is based on the molecular weight of glutamine rather than its amino nitrogen content. Since glutamine contains only 9.59 percent amino nitrogen the true amino nitrogen values are only 9.59 percent of those reported.

Experimental Results

Curly top was moderately severe at Jerome, Idaho, in 1958. This degree of infection was expected because the experimental plot adjoined the desert breeding area of the beet leafhopper (*Cerculifer tenellus*). On August 1, 94 percent of the Ovana fodder beets were obviously affected by the disease. In contrast only 15 percent of the F_1 hybrids (US 35 $\times$ Ovana) and still smaller percentages of the other varieties were obviously affected. By harvest time all the Ovana fodder beets were severely injured but since the infection came late in the season most of the beets survived and a yield of 13.1 tons per acre was produced.

Curly-top exposure at Salt Lake City was rather severe but even later in the season than at Jerome. At harvest all Ovana fodder beets were injured so that they yielded only 15.9 tons per acre. At Salt Lake City some F_1 hybrids showed mild curly-top symptoms before harvest in 1958 but apparently yielded nearly normally.

Yield and sugar percentage

At Jerome, Idaho, all the F_1, b_1, and b_2 hybrids produced yields in excess of 30 tons per acre (Table 2). The yield of 27.3 tons per acre and 18.7 percent sugar by US 35 is considered an excellent yield. US 35 produced 9,995 pounds of sugar per acre. However, SL 630, with a yield oof 33.2 tons and 18.3 percent sugar, yielded 11,865 pounds of sugar per acre. The low yield

Table 2.—Second-generation hybrid SL 630 compared with the F_1 and b_1 hybrids and with parental varieties US 35 and the Ovana fodder beet at Jerome, Idaho, and Salt Lake City, Utah, 1958.

Current S.L. number	Description	Acre Yield: Sugar	Acre Yield: Tons	Sucrose	Purity	Amino N	Na	K	Curly-top August 1	Beets per 100-feet of row
		Pounds	Beets	Percent	Percent	Percent	ppm	÷ 10	Percent	
		Jerome, Idaho—8 replications each								
308	Ovana	3,305	13.1	13.1	89.8	.33	54	222	94.1	86
308+5	F_1 (US 35 × Ovana)	9,915	32.1	15.9	92.0	.34	29	248	14.8	98
431+5	b_1 [US 35 × (US 35 × Ovana)]	10,510	31.4	17.1	92.6	.31	23	228	9.9	105
024	US 35	9,995	27.3	18.7	92.3	.32	16	194	7.9	100
630	(b_1 × CT8)	11,865	33.2	18.3	94.1	.34	19	174	3.5	96
	LSD 5%	1,040	3.19	0.54	0.97	NS	5	34		
		Salt Lake City, Utah—20 replications each								
308	Ovana	2,939	15.9	8.4	70.6	.92	267	294	Severe[1]	71
308+5	F_1 (US 35 × Ovana)	9,282	41.7	11.1	79.4	.83	129	399		109
431+5	b_1 [US 35 × (US 35 × Ovana)]	9,652	38.3	12.7	81.2	.90	100	407		108
024	US 35	9,468	32.8	14.4	83.8	.82	86	415		104
630	(b_1 × CT8)	12,084	40.9	14.8	85.4	.69	73	372		105
	LSD 5%	856	3.59	0.56	1.60	.08	18	18		

[1] At Salt Lake City nearly all Ovana plants were badly injured by curly top. In the F_1 hybrids very mild symptoms were evident on most plants, but this degree of infection did not appear to interfere with growth. Mild curly-top symptoms were evident on only occasional plants of the other three more highly resistant varieties.

of 13.1 tons per acre and abnormally high sugar percentage of the Ovana fodder beets may be explained by the effect of curly top. At harvest most of the leaves were dead and the roots were somewhat desiccated.

Yields were all higher and sugar percentages were lower at Salt Lake City than at Jerome, Idaho. Hybrid SL 630, in spite of its extremely high yield, had a sugar percentage of 14.8 percent compared with 14.4 percent for variety US 35. The difference of 0.4 percent sugar is not statistically significant but the fact that the high-yielding beets of hybrid SL 630 were equal to that of the sugar type variety US 35 was surprising and rather remarkable. The sugar per acre yield for SL 630 was 12,084 pounds in comparison with 9,468 pounds for US 35. This difference is highly significant and amounts to an increase of 28 percent over that of US 35.

In 1959 unreplicated plots of SL 630, SL 631, and Ovana were planted at Salt Lake City. The data obtained confirmed earlier replicated tests. SL 630 indicated a yield of 12,760 pounds of sugar per acre in comparison with 11,184 pounds for SL 631. Curly-top exposure was very light in 1959. Under these conditions and lacking competition the Ovana variety yielded 78 tons per acre with 5.7 percent sugar for a gross sugar yield of 8,960 pounds per acre.

Purity and components of purity

The hybrid SL 630 was significantly superior in purity to the sugar-type variety US 35 at both locations under widely different conditions of salinity and nitrogen fertility. This high purity of hybrid SL 630 may be partly explained by considering some of the individual components of impurity (6). Differences in the three individual components of impurity were not statistically significant at Jerome, but at Salt Lake City under both high salinity and nitrate fertility which depressed both sugar percentage and quality of all varieties SL 630 was quite superior. Both amino nitrogen and potassium content were significantly lower in SL 630 than in US 35. Although the difference in sodium content was not significant it favored SL 630 over US 35.

Discussion

Unless one considers the components of impurity individually, it would seem incredible that the low-purity Ovana variety should have contributed anything toward improved purity in its hybrid offspring. Undoubtedly a very significant factor was the highly superior beet selected in the b_1 parental line (Table 1). Since CT8 hybrids have not been particularly outstanding in previous hybrid combinations, the superiority of SL 630 might be con-

sidered a rather specific case of genetic heterosis for quality factors as well as for yield.

Previous studies of fodder beets and half-sugar beets have shown them to be low in respiration rate (10). The respiration-rate studied on individual beets in 1959 confirmed this general observation for the Ovana variety. The Ovana fodder beet was extremely low in respiration rate. This fact might be explained on the basis of its very low sugar percentage, but three one-eighth-Ovana hybrids, including SL 630 and SL 631, were also low in respiration in comparison with the sugar beet variety with which they were compared. There is little doubt that the low respiration rates of the Ovana hybrids were inherited from the Ovana rather than due to low sugar percentage. Respiration rates between 70 and 90 mg of CO_2 per kg per hour are usual for sugar beets (10). In the 1959 tests the rates for US 41, Ovana, and the three one-eighth-Ovana hybrids were 70, 50, and 60 to 62 mg CO_2 per kg per hour, respectively.

Development of the hybrid SL 630 may therefore be considered an approach to the breeding of superior-quality and high-yielding sugar beets. Valuable information may be lost by considering purity determinations alone without information on some of the respective components of purity. Furthermore, the determinations for amino N, Na, and K may be more accurate than purity determinations because errors in sugar as well as dry-substance determinations are involved in the purity values. The ease and rapidity of the amino N, Na, and K determinations, and the fact that they may be made with less than 20 ml of the same clarified filtrate used for sugar analysis, make the separate determinations especially well adapted for breeding and selection work (10). If the trend toward excessive use of nitrogen fertiliers ccontinues (2, 11), selection work for low amino N and other constituents of impurity in the beet root should warrant extensive investigation because it has been shown (11) that a high uptake of nitrate increases the uptake of Na and K and thus decreases the purity of the sugar beet and increases the difficulties of sugar extraction (7).

Summary

A high-yielding curly-top-resistant sugar beet hybrid with relatively high sugar percentage and exceptionally high purity was developed by utilizing the Ovana fodder beet in the parentage. The selection of a highly superior beet made in the b_1 generation hybrid US 35 $\times$ (US 35 $\times$ Ovana) for high root weight, high sugar percentage, low amino nitrogen, low Na, and low K was undoubtedly responsible for the superior performance of

SL 630. These selected b_1 beets were then crossed to the inbred CT8. All crossing was effected by the use of mendelian male sterility. The final selected hybrid was higher in curly-top resistance than either of the two curly-top-resistant parents US 35 or CT8. This high degree of curly-top resistance and the remarkable yield and low respiration rate may be largely attributed to hybrid vigor and high yield prepotency derived from the Ovana fodder beet. The Ovana fodder beets were very high in yield and sodium content. They were very low in sugar and respiration rate. The selection in the b_1 generation and hybridization to the high-sugar, high-purity inbred CT8 produced a hybrid that combined the good qualities of all three parents.

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Artificial Exposure of Sugar Beets to Rhizoctonia Solani[1]

VICTOR G. PIERSON AND JOHN O. GASKILL[2]

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Introduction

Root rot caused by *Rhizoctonia solani* Kuehn, also known as *Pellicularia filamentosa* (Pat.) Rogers, is a serious disease of sugar beets for which satisfactory control measures are not known. Attempts to breed Rhizoctonia resistant varieties have been impeded by the lack of satisfactory techniques for creating artificial exposure to attack. Such techniques must provide for a high degree of uniformity in the intensity of exposure to the pathogen and also for maintenance of exposure intensities at levels suitable for root selection and progeny testing purposes, respectively. Several studies of inoculation methods have been reported [Houston (8)[3], LeClerg (10 and 11), Kreitlow and Sherwin (9), Erwin (5), and Schuster et al. (14)], but none has been shown by experience to be entirely satisfactory for such purposes. The relation of soil amendments to Rhizoctonia attack of sugar beets has been studied by several authors [Blair (2), Boosalis (3), Dunleavy (4), Holst and Cormany (7), and Sanford (12 and 13)].

Materials and Methods

Twenty-five *R. solani* isolates from Colorado crop plants and soil were tested for pathogenicity to sugar beets at 3 stages of seedling development. Three, replicated, greenhouse pot experiments were involved in this study, each experiment including the full set of 25 isolates. Steamed and non-steamed soils were used in separate experiments. Certain isolates did not attack the plants, and the pathogenic action of others ranged from mild to very severe. Isolates classed as pathogenic, on the basis of these results, were used selectively in all field and greenhouse inoculation experiments initiated during 1958. In earlier inoculation experiments in the field, the inoculum used consisted of a composite of 18 Rhizoctonia isolates of undetermined pathogenicity.

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[2] Formerly Graduate Research Assistant, Colorado Agricultural Experiment Station, and Plant Pathologist, U.S. Department of Agriculture, respectively. Acknowledgment is made to L. W. Durrell, Dean Emeritus, College of Science and Arts, Colorado State University, for assistance and suggestions in conducting this study.

[3] Numbers in parentheses refer to literature cited.

For field experiments, inoculum was prepared by growing Rhizoctonia isolates for 2 to 3 weeks, at approximately 25° C, in flasks containing moist, sterilized, whole grain. The cultures then were dried in a mechanically ventilated oven at approximately 40° C. Except where otherwise indicated, the dried material was ground in a Wiley mill, passing through a 2-mm wire mesh screen. Except where the use of sorghum grain is indicated, barley grain was used as the substrate for the preparation of all inoculum for field use. For check purposes in certain experiments, the fungus in ground grain inoculum was killed by means of propylene oxide, alone or in conjuction with autoclaving. In some cases autoclaved substrates (ground grain) also were used for check purposes.

Sugar beet strains or varieties used in these investigations were as follows:

A. SP 52108-0: A USDA variety resistant to Cercospora leaf spot (*C. beticola* Sacc.) and to black root of the type caused by *Aphanomyces cochlioides* Drechs.
B. SP 581815-022: B-590♀ × miscellaneous material selected at Fort Collins for Rhizoctonia resistance. The ♀, furnished by The Great Western Sugar Company, had resulted from selection for Rhizoctonia resistance.
C. SP 471001-0: A vigorous inbred, susceptible to Cercospora leaf spot and to Aphanomyces-type black rot.
D. Kleinwanzleben-E: A commercial European variety.
E. SP 5832-0: A monogerm USDA variety, resistant to leaf spot and black root.
F. GW 359: A leaf spot resistant commercial variety widely grown in northern Colorado in recent years. Seed of this variety was used in all experiments reported in this article where the variety is not specifically indicated in the presentation of results.

On the basis of 1957 results at Fort Collins (unpublished), strains C and D were considered quite susceptible to *R. solani* and A and B were thought to have at least some degree of resistance or tolerance to that pathogen. In all experiments involving the application of inoculum in the seed furrow with the seed at time of planting, the seed was treated before planting with an ample quantity of 70 percent maneb dust, the excess removed by vigorous screening. Maneb seed treatment also was used in all other experiments, sometimes in combination with Dieldrin for root maggot control.

All field experiments were conducted on the Hospital Farm near Fort Collins, Colorado. Soil type in the area used for the

experiments is classed as Fort Collins fine sandy loam or Fort Collins loam, light-textured phase. The sprinkler method of irrigation was used exclusively.

Two principal inoculum application methods were tested under field conditions. They involved the placement of ground grain inoculum, respectively: 1) with the seed at planting, and 2) in contact with the sugar beet tap root after thinning. In the first case, a measured amount of sugar beet seed was placed on the seed belt of a drill, and a measured quantity of inoculum was spread uniformly over the seed[4]. The mixture then was drilled the full length of 1 plot row. With the second method, the soil was removed from around the tap root to a depth of 1 to 1¼ inches, a measured quantity of inoculum was placed in contact with the tap root, and the soil was pulled back into its original position (Figure 3). A third method of inoculation, used in a preliminary field trial, involved the application of ground grain inoculum in the center of the foliar rosette, after thinning, allowing it to fall on the surface of the soil at will.

Figure 3.—Post-thinning method of applying Rhizoctonia inoculum to sugar beet plants. The completed job is shown for plant at left where the inoculum is covered with soil.

A preliminary field experiment, dealing with the effects of soil amendments on Rhizoctonia attack, was conducted in 1958. Each of 2 amendments, yellow corn meal and dried beet pulp[5], was applied at 2 rates: 0.2 and 0.4 pound per 17 feet of row. The

[4] This method was suggested by N. R. Gerhold and K. E. Mueller, while serving as Associate Plant Pathologist and Junior Plant Pathologist, respectively, Colorado Agricultural Experiment Station.

[5] LPC dried pulp made by The Great Western Sugar Company, containing the equivalent of 14 percent crude protein.

amendment was distributed in a band approximately 6 inches wide, immediately before planting, and worked into the soil to a depth of about 3 inches. The beet row was planted approximately 1 inch to the left of the center line of the 6-inch amendment band, using a belt-type planter and a uniform seeding rate. On July 29, 3 weeks after planting, living or killed ground-barley (composite Rhizoctonia) inoculum was applied to the right of the center line of the amendment band, approximately 2 inches from the beet row and 1½ inches deep. There were 2 blocks of plots, each plot consisting of 1 row, 17 feet long. The 0.2-pound soil amendment rate was used in block 1; the 0.4-pound rate in black 2. Appropriate soil-amendment and inoculum checks were included in each block.

Two experiments were designed to study the relationship of plant ages and fungus isolates to disease reaction of 4 sugar beet strains growing in an artificial medium in the greenhouse. Sugar beet strains A, B, C, and D were used. All seed was sized between 10/64 and 15/64 inch and planted in a 4.5-inch diameter circle in 6-inch pots. Three Rhizoctonia isolates (B-6, B-12, and S-10), rated respectively as strong, moderate, and weak in pothogenicity, plus check constituted 4 so-called "isolate" treatments. The inoculum consisted of 1 cm mycelial agar disks, and on December 30, 1958, one disk was placed ½ inch deep in the center of each pot to be inoculated. A steamed mixture of 50 percent Canadian peat moss and 50 percent fine, washed, river sand, by volume, as proposed by Baker and coworkers (1), was used as a growth medium. Nutrients supplied in the watering system solution, in parts per million, were as follows: N = 129.4, Na= 8.0, K_2O = 132.0, Mg = 12.0, SO_4 = 48.0, B_2O_3 = 0.5, and P_2O_5 = 35.0. Calcium was incorporated in the sand-peat moss mixture at the rate of 5.7 grams of powdered calcium carbonate per gallon. Greenhouse air temperatures, as recorded by thermograph, were approximately as follows: 6:30 PM to 8:00 AM, 16° C; 9:30 AM to 4:30 PM, 27° C; over-all, 21° C. Soil temperatures, in the pots, averaged approximately 14° and 26° C at 8:00 AM and 4:00 PM, respectively.

In the first of these 2 greenhouse experiments, planting was done on November 13 and December 6, and populations were thinned to 10 plants per pot prior to inoculation. All possible combinations of 4 sugar beet strains, 2 age classes, and 4 isolate treatments resulted in a total of 32 entry numbers for the experiment. A randomized complete block design was used with 3 replications. In the second greenhouse experiment, planting was done immediately before inoculation was performed. Seeding

rates were adjusted according to germination in an attempt to provide 30 potential seedlings per pot. All possible combinations of 4 sugar beet strains and 4 isolate treatments resulted in 16 entry numbers. The pots were arranged in a randomized complete block design with 5 replications.

Additional details regarding materials and methods are given in the report of results for individual experiments.

Experimental Results and Discussion

Inoculum applied with the seed

In a preliminary field experiment with 3 replications, in 1957, ground barley grain (Rhizoctonia) inoculum was applied with the seed at approximately 0.4, 0.8, 1.6, and 3.2 ml per foot of row. Stands for those treatments after thinning, expressed as percentage of the non-inoculated check, were 7, 6, 2, and 0, respectively. In a comparison of ground- and whole-barley inoculum, applied at the rate of 0.4 gram per foot of row, pre-thinning stands were 1 and 28 percent of check, respectively. The greater percentage of killing obtained for the ground inoculum was attributed to more uniform distribution of infective Rhizoctonia units. Where dry, ground, autoclaved barley grain was applied at the rate of 0.4 gram per foot of row, pre-thinning stand was 7 percent of check.

Table 1.—Effects of type and rate of application of Rhizoctonia inoculum, with the seed, on sugar beet stand under field conditions.

Treat. number	Inoculum			Isolates and living plants per plot		
	Substrate	Kind	Amount per ft.	B-7[a]	S-21[a]	Average
			Ml	No.	No.	No.
Comparison of rates:						
1	barley	living Rhizoc.	0.03	30.3	20.7	25.5
2	do	do	0.12	3.0	3.0	3.0
3	do	do	0.29	2.0	3.7	2.8
6	sorghum	do	0.03	22.7	48.3	35.5
7	do	do	0.12	4.0	20.7	12.3
8	do	do	0.29	1.0	8.3	4.7
LSD (5-percent point)				11.1	11.1	
F-value for interaction, isolates × treatments = 5.67**						
Comparison of "checks":						
4	barley	killed Rhizoc.[b]	0.12	96.3	124.0	110.2
5	do	ster. substrate	0.12	33.7	47.0	40.3
9	sorghum	killed Rhizoc.[b]	0.12	96.0	95.3	95.7
10	do	ster. substrate	0.12	50.0	63.7	56.8
11	none	----------	------	124.7	121.0	122.8
LSD (5-percent point)				25.0	25.0	17.7
F-value for intraction, isolates × treatments = 1.14						

[a] Three-plot averages; counts made in 14 feet of row per plot, 9/19/58, 53 days after planting.
[b] Killing performed by means of propylene oxide.
** F-value exceeds the 1-percent point.

In 1958, ground grain inoculum was applied with the seed, in a field experiment, at 0.03, 0.12, and 0.29 ml per foot of row. Killed Rhizoctonia inoculum and sterilized substrates were applied at the rate of 0.12 ml per foot. A split-plot design was used with main plots (Rhizoctonia isolates) composed of sub-plots of inoculum types and rates. There were 3 randomized complete blocks. The results from this experiment are presented in Table 1 and in Figures 1 and 2. As shown in Table 1, the 2 heavier rates of application of living inoculum resulted in extremely severe reductions in stand for each type of substrate. Stand reductions for the 0.03-ml rate were significantly less severe and probably much nearer the point that would be most suitable for progeny testing purposes. Where barley-substrate inoculum was used at the 0.03-ml rate, the average number of surviving plants per plot (25.5) was 21 percent of that shown for the non-treated check, treatment no. 11. The corresponding percent-of-check value for the 0.03-ml rate of sorghum-substrate inoculum was 29. It is recognized that such a degree of exposure to the pathogen probably would permit the survival of many "escapes." Con-

Figure 1.—Comparison of 3 rates of application of living Rhizoctonia inoculum, with sugar beet seed, at time of planting. Plots with large labels, left to right, received 0.03 ml, 0.12 ml, and 0.29 ml, respectively, per foot of row.

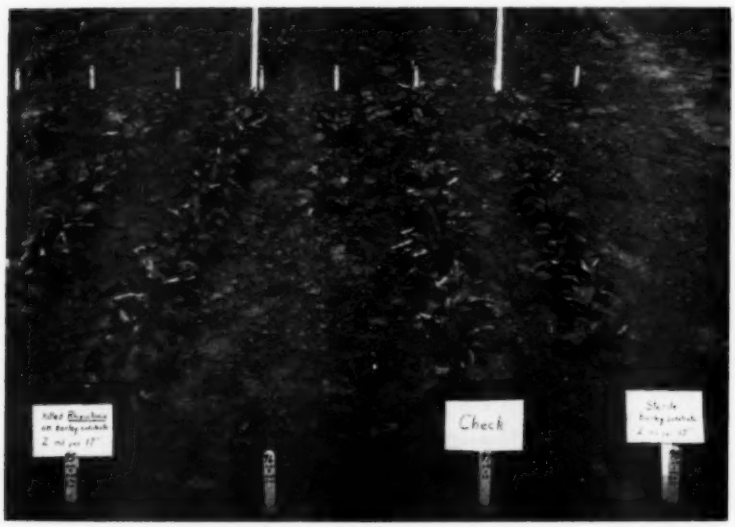

Figure 2.—Effects of killed Rhizoctonia inoculum and sterilized barley substrate, applied with the seed, in comparison with the non-treated check. Labeled plots at left and right received killed inoculum and sterilized substrate, respectively, at the rate of 0.12 ml per foot of row.

sequently, if such a method of inoculation were to be used for individual plant selection purposes, a much more consistent or uniform exposure would be required. For such purposes, and under the conditions of this experiment, it appears that an inoculum application rate of 0.29 ml per foot of row, or slightly higher, would be desirable. The relative pathogenicity of the fungus isolate also must be considered, of course. It is conceivable that a much heavier application rate, using an isolate of only medium pathogenicity, would be more suitable for identification and preservation of individual plants with a modicum though desirable degree of seedling resistance to Rhizoctonia, if such plants exist.

Certain comparisons among "check" treatments are of interest (Table 1). The average stand for each of the treatments involving the application of killed-Rhizoctonia inoculum (treatments 4 and 9) was below that obtained for the basic check treatment (no. 11), and in one case the difference was quite significant. The average stand for each of the sterilized-substrate treatments (nos. 5 and 10), in turn, was far below the average for the corresponding killed-inoculum treatment, both differences being highly significant.

The role of one type of sterilized substrate in promoting damping-off among young seedlings was studied in a preliminary laboratory experiment. Field soil was used without steaming or other fungicidal treatment. Seed was planted in small glass-walled containers, with and without dry, ground, autoclaved barley grain, and the containers then were held at a temperature of 28° C. At intervals of 72 and 96 hours after planting, seedlings were removed from the soil and plated on nutrient agar. The fungi obtained were identified as to genera. The highlight of this experiment was the behavior noted for *Pythium* sp. Seedlings from 5 of 32 seed balls of the check treatment produced *Pythium* sp. on nutrient agar. In contrast, seedlings from 18 of 32 seed balls, planted in containers receiving the sterilized substrate, produced *Pythium* sp. Species of *Pythium* are known to be quite pathogenic to young sugar beet seedlings, and one species has been reported as the chief cause of pre- and post-emergence damping-off in a series of seed treatment experiments conducted in the vicinity of Fort Collins a number of years ago (6). Admittedly the seedling populations involved in this experiment were not large, and soil conditions in the laboratory differed from field conditions. However, the results seem to justify the tentative conclusion that the severe stand losses observed, where sterilized barley-type substrate had been applied with the seed at time of planting, may be attributed largely to stimulation of Pythium activity by the presence of this readily available food material.

Inoculum applied after thinning

Two dates of post-thinning sub-surface inoculation were compared in a preliminary field experiment, with 3 replications, in 1957. Thinning was performed on July 29 at about the usual stage of plant development. Ground barley grain inoculum, composed of a mixture of Rhizoctonia isolates, was applied at the rate of 0.6 gram per plant. Dates of inoculation compared were August 2 and August 15. Living plants in the inoculated plots were counted at time of inoculation and again at harvest, October 21. At harvest, each living plant was classified for disease reaction, as illustrated in Figure 4, and the total weight of roots of living plants was determined for each plot. In the non-inoculated check plots, no loss in stand occurred between thinning and harvest, the average disease rating at harvest was 1.2 (essentially healthy), and the total weight of roots per plot was 36.6 pounds. For plots inoculated on August 2 (4 days after thinning), a 32-percent loss of stand occurred between time of inoculation and harvest, and the average disease rating and total

weight of roots per plot were 3.2 and 19.6 pounds, respectively. Corresponding averages for plots inoculated 17 days after thinning were 2 percent, 2.2, and 36.1. It seems clear that delaying the inoculation 13 days resulted in a decrease in severity of attack. However, not all of this difference can be attributed to plant age effects.

Figure 4.—Rhizoctonia disease ratings.

The relation of plant age to Rhizoctonia attack in the field was studied further in 1958, using the post-thinning sub-surface method of inoculum application, 4 dates of planting, and a single inoculation date. A split-plot design with 4 randomized complete blocks was used. Plant ages constituted the main plots. Each main plot was composed of 3 sub-plots which received living inoculum, killed inoculum, and no inoculum, respectively. Ground barley inoculum, composed of a mixture of 13 pathogenic isolates, was used at the rate of 2 ml per plant. At the time of inoculation (August 1), soil adjacent to all plants in the check plots was manipulated in exactly the same manner as in the inoculated plots. Stand counts made on August 5 represented numbers of plants living at time of inoculation. The lower initial stand counts in age class IV (Table 2) were the result of removal of curly top infected individuals just prior to inoculation and are considered unimportant insofar as the outcome of this experiment is concerned. As shown in Table 2 and Figure 5, there was a strong relationship between plant age and severity of Rhizoctonia attack, where living inoculum was applied, the older plants tending to die later and exhibiting a higher percentage survival at harvest. Effects of killed Rhizoctonia inoculum were

Table 2.—The relation of sugar beet age to severity of Rhizoctonia attack under field conditions[a].

Inoculum	Plant age symbol[b]	No. of days, thinning to inoculation[c]	No. of plants per plot 8/5	Surviving plants		Disease rating 9/27[e]
				Percent[d]		
				8/19	9/27	
Living Rhizoc.	I	0	27.0	5.9	0.0	—
	II	11	29.3	8.6	0.8	5.0
	III	22	25.3	83.0	2.1	4.5
	IV	44	18.5	98.6	26.9	4.5
Killed Rhizoc.[f]	I	0	29.5	100.0	97.8	1.2
	II	11	32.3	100.0	100.0	1.1
	III	22	26.8	100.0	100.0	1.3
	IV	44	16.3	100.0	97.7	1.2
Check	I	0	30.3	100.0	100.0	1.1
	II	11	31.0	99.2	99.2	1.1
	III	22	23.5	100.0	100.0	1.1
	IV	44	19.0	100.0	100.0	1.0

[a] Data presented as 4-plot averages; each plot, 2 rows × 12 feet (Counted area, 2 rows × 11 feet).
[b] Planting and thinning dates were as follows: I = 7/3, 8/1; II = 6/27, 7/21; III = 6/13, 7/10; IV = 5/22, 6/18; respectively.
[c] Inoculation performed 8/1/58.
[d] Percentages based on initial stand (8/5).
[e] Basis of ratings: 1 = essentially healthy; 5 = very severe disease.
[f] Killing performed by means of propylene oxide plus autoclaving

negligible. These results indicate that, in a Rhizoctonia resistance breeding program, the intensity of exposure to the pathogen may be regulated to some extent by suitable timing of inoculation with respect to plant age.

Rates of post-thinning inoculum application, sub-surface, were studied in a replicated experiment in the field, in 1958, using 1-, 2-, and 4-ml amounts of ground-barley inoculum per plant. The 13-isolate composite, mentioned in the preceding paragraph, was employed. Killed inoculum and sterilized substrate were applied at the rate of 2 ml per plant. Sugar beet strains C and E were used. Disease effects were negligible in all plots receiving killed inoculum, sterilized substrate, or no inoculum or substrate of any kind. Of 514 plants inoculated with living Rhizoctonia 4 days after thinning, only 2 were alive 45 days later, and both were severely diseased. One of those plants had received 1 ml of inoculum and the other had received 2 ml. These results do not offer any encouragement for control of intensity of exposure to Rhizoctonia, through regulation of amount of inoculum, where the post-thinning sub-surface method is employed. However, further study, involving lower inoculum dosages, is needed before a definite conclusion can be reached in this regard.

A preliminary field trial, with inoculum applied in the petiole-crown region, was conducted in 1958, using 1-, 2-, and

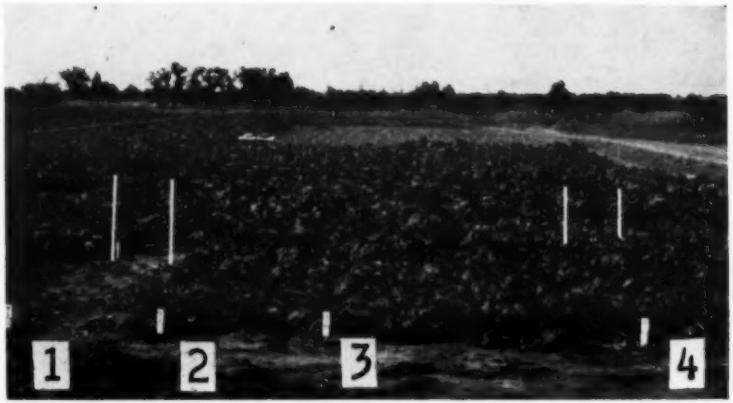

Figure 5. — Influence of plant age on Rhizoctonia attack. Plots 1 and 4 (2-row plots marked at far end with tall white stakes) were planted on June 27 and 13, respectively, and both had received living Rhizoctonia inoculum on August 1, 1958, 12 days before picture was taken.

Table 3.—The influence of soil amendments on Rhizoctonia attack of sugar beets in the field.

Inoculum	Soil amendment and living plants per plot[a]								
	No amendment			Beet pulp			Corn meal		
	Initial stand[b]	Final stand[c]		Initial stand[b]	Final stand[c]		Initial stand[b]	Final stand[c]	
		Surv.	Dis.		Surv.	Dis.		Surv.	Dis.
	No.	Pct.	Pct.	No.	Pct.	Pct.	No.	Pct.	Pct.
Living Rhizoc.	143.5	85.7	40.6	56.5	98.2	5.5	69.5	97.1	17.9
Killed Rhizoc.[d]	133.0	92.9	0.0	88.5	98.9	0.0	62.0	100.0	0.0
None	117.5	97.0	1.4	75.5	100.0	0.0	48.5	100.0	0.0
Average	131.3			73.5			60.0		

[a] Basic data presented as 2-plot averages, each plot 1 row × 17 feet; counts, 14 feet of row per plot.
[b] Initial stand counts 8/2/58; inoculum applied 7/29, 21 days after planting.
[c] Final stand counts 9/17; percentage survival based on initial number of plants; percentage diseased based on number surviving.
[d] Killing performed by means of propylene oxide plus autoclaving.

4-ml rates. Of a total of 128 plants of sugar beet strain C, inoculated with composite living Rhizoctonia 4 days after thinning, all were dead 45 days later. There was no loss due to Rhizoctonia in the non-inoculated check plots. Thus, the use of inoculum in this manner appears rather promising as an inexpensive and effective means of exposing large populations of sugar beets to Rhizoctonia under field conditions. Whether the intensity of exposure to the pathogen can be controlled satisfactorily, and whether exposure in this manner is desirable in other respects, must be determined by further experimentation.

Soil amendment trial

The results of a preliminary soil-amendment experiment, conducted under field conditions, are shown in Table 3. Since results for the 0.2- and 0.4-pound rates were not contradictory, the 2 rates were combined in the preparation of Table 3. In studying these data, it should be noted that they are based on 2 replications, only. However, certain treatment effects or trends are of such magnitude that they appear to be quite meaningful.

It was observed in the field that damping-off was severe in those plots receiving either of the soil amendments and negligible in plots receiving no soil amendment. Since the initial stand counts, shown in Table 3, were made before killing due to the application of Rhizoctonia inoculum had begun, the effects of soil amendments on damping-off, independent of the effects of inoculation, may be observed in the initial stand columns. Average initial stand for the 6 plots given no soil amendment was 131.3 plants per plot, and the comparable averages for the beet pulp and corn meal amendment treatments were 73.5 and 60.0, respectively. Thus, the early losses in stand, indicated by these results and attributed to the respective amendments, were 44 and 54 percent. It is assumed that those losses were primarily due to stimulation, by the amendments, of the activity of pathogenic organisms occurring naturally in the soil.

Stand losses occurring during the 46-day period, between the dates when the initial and final counts were made, provide a basis for appraising effects of inoculation with Rhizoctonia and counter effects of soil amendments. As shown in Table 3, survival percentages ranged from 92.9 to 100.0 for the 2 types of checks (killed Rhizoctonia inoculum and no inoculum), with no obvious effects attributable to soil amendments or to the use of killed inoculum. Where living Rhizoctonia inoculum was used, survival percentages for no-amendment and for beet pulp and corn meal amendments, respectively, were 85.7, 98.2, and 97.1.

Table 4.—The influence of sugar beet strain and fungus isolate on Rhizoctonia attack under controlled conditions; inoculum applied after thinning.

Isolate	Sugar beet strain	Number of living plants per pot[a]										
		Total										Healthy
		1/26	2/2	2/9	2/16	2/23	3/2	3/9	3/16	3/23	3/31	3/31
B-6	A	10.0	7.0	5.8	3.2	1.2	0.0	0.0	0.0	0.0	0.0	0.0
	B	10.0	7.7	7.5	3.3	0.5	0.0	0.0	0.0	0.0	0.0	0.0
	C	10.0	6.2	4.8	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	D	10.0	8.7	7.3	3.8	1.7	0.2	0.0	0.0	0.0	0.0	0.0
B-12	A	10.0	10.0	10.0	9.8	9.7	9.3	8.8	8.3	7.8	5.2	1.0
	B	10.0	10.0	10.0	10.0	10.0	10.0	9.5	9.2	9.2	5.7	0.8
	C	10.0	10.0	10.0	10.0	10.0	9.8	9.8	9.0	7.8	6.0	0.5
	D	10.0	9.8	9.8	9.8	9.7	9.7	9.2	9.0	6.8	4.7	0.8
S-10	A	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
	B	10.0	10.0	10.0	10.0	10.0	10 0	10.0	10.0	10.0	10.0	10.0
	C	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
	D	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Check	A	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
	B	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
	C	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
	D	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0

[a] Data presented as 6-pot averages (6-inch pots). Three replications were planted on November 13, and the other 3 on December 6, 1958. Inoculation was performed on December 30.

Table 5.—The influence of sugar beet strain and fungus isolate on Rhizoctonia attack under controlled conditions; inoculum applied at time of planting[a].

Isolate	Sugar beet Strain	Total no. emerged plants per pot	Living plants as percent of total emergence[b]											
			Total											Healthy
			1/12	1/19	1/26	2/2	2/9	2/16	2/23	3/2	3/9	3/23	4/1	4/1
B-6	A	34.2	97.8	86.5	68.3	45.5	35.5	18.0	9.5	3.2	0.0	0.0	0.0	0.0
	B	30.2	96.2	77.1	57.7	39.7	30.2	19.5	12.4	4.9	1.2	0.0	0.0	0.0
	C	34.2	93.1	80.8	69.4	43.4	31.5	13 7	4.4	2.2	0.0	0.0	0.0	0.0
	D	28.6	97.6	81.2	69.9	53.7	47.1	35 8	15.7	9.4	2.1	0.0	0.0	0.0
B-12	A	32.0	97.8	99.4	100.0	99.2	99.2	99.2	98.6	98.6	98.6	93.9	66.6	11.7
	B	32.4	98.5	97.5	98.9	98.9	98.9	98.9	98.9	98.9	97.4	96.3	73.2	21.5
	C	33.8	92.3	98.2	100.0	100.0	100.0	98.3	96.4	94.2	88.4	84.1	57.0	11.2
	D	30.8	99.4	100.0	100.0	99.4	99.4	98.0	98.0	95.2	93.3	89.3	61.5	11.1
S-10	A	31.2	99.4	100.0	100.0	100.0	100.0	99.4	99.4	99.4	99.4	99.4	98.7	98.7
	B	28.2	97.1	99.4	100.0	99.4	98.6	97.4	97.4	97.4	97.4	97.4	95.5	94.9
	C	39.4	96.4	98.8	100.0	100.0	99.6	99.6	99.6	99.6	99.6	99.6	98.6	95.1
	D	28.6	99.3	98.9	98.9	98.9	98.9	98 9	98.9	98.9	98.9	98.9	98.3	94.7
Ck.	A	31.0	98.7	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.4	99.4
	B	31.8	99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4
	C	33.0	93.4	98.7	98.7	99.2	100.0	100.0	100.0	100.0	100.0	100.0	99.0	99.0
	D	30.0	98.1	98.7	98.0	98.6	99.3	99.3	99.3	99.3	99.3	99.3	98.6	98.6

[a] Planting and inoculation performed on December 30, 1958.
[b] Data presented as 5-pot averags (6-inch pots).

Corresponding percentages of diseased plants at harvest were 40.6, 5.5, and 17.9, respectively. It seems clear that the living inoculum caused substantial injury in the no-amendment plots, and it is tentatively concluded that the amendments, where applied, provided some degree of protection. It is assumed that this protective influence was due to stimulation of soil microorganisms detrimental to Rhizoctonia. This apparent protective effect of the amendments was in agreement with results reported by Sanford (13) and Dunleavy (4), but contrary to the observations of Holst and Cormany (7).

Inoculation of plants growing under controlled conditions

The reaction of 4 sugar beet varieties or strains to 3 Rhizoctonia isolates, in a sand-peat moss medium in the greenhouse, is shown in Tables 4 and 5. Since there was very little difference in reaction of the 2 older seedling groups, the results for those groups were combined (Table 4). Results for the youngest seedling group—the group that was inoculated at time of planting—are presented in Table 5. Isolate B-6 killed all seedlings of all sugar beet strains and ages before or during the week of March 9—i.e., within approximately 10 weeks after inoculation. The final killing action occurred within a 3-week period for all strains. Isolate B-12 was much slower in its attack than isolate B-6, and 13 weeks after inoculation it had not killed all the plants of any strain. Isolate S-10 appeared to be almost nonpathogenic under these conditions. It exhibited very little killing and disease in the younger beets, and none in either of the older sets.

Some evidence that sugar beet strain C was more susceptible to Rhizoctonia than the other 3 strains, under the conditions of this experiment, may be found in Tables 4 and 5. That evidence is rather meager and cannot be considered as conclusive. Differences among the other 3 strains appeared to be negligible.

The preparation of a reproducible soil mixture, suitable for Rhizoctonia resistance testing purposes in the greenhouse, repreents a substantial problem. It is believed that the sand-peat moss medium used in this study appears rather promising as a solution to this problem.

Summary

A series of greenhouse and field experiments was conducted for the purpose of devising techniques for exposing sugar beets to *Rhizoctonia solani* suitable for disease resistance testing and selection purposes.

Ground and whole barley-grain Rhizoctonia inocula, in dry form, were compared and found to produce relatively even and

sporadic attack of sugar beet seedlings, respectively, when applied with the seed at identical rates. The ground, barley-grain inoculum, prepared from virulent isolates and applied with the seed at time of planting in field plots at the rate of 0.03 ml per foot of row, produced a degree of exposure to the pathogen considered satisfactory for progeny testing purposes.

Where ground, barley-grain inoculum was applied in contact with the tap root and below the soil surface at the rate of 2 ml per plant, from 0 to 11 days after thinning, less than 1 percent of the plants were alive at harvest. Of comparable older plants, inoculated 44 days after thinning, 27 percent survived. Attempts to control intensity of Rhizoctonia exposure by varying the rate of post-thinning application of inoculum were unsuccessful.

Four sugar beet strains, thought to differ somewhat in resistance or tolerance to *R. solani*, were tested under controlled conditions against *R. solani* isolates with past histories of strong, moderate, and weak pathogenicity, respectively. The strains were tested at 3 stages of seedling development. Differences among strains, in apparent resistance, were rather small and inconclusive. However, the relatively consistent pathogenic activity of the Rhizoctonia isolates indicated that the sand-peat moss medium employed in this study may be a useful tool for Rhizoctonia resistance testing and selection work in the greenhouse.

Beet pulp and corn meal, incorporated with field soil immediately prior to planting, caused a severe, early reduction in sugar beet seedling stand where artificial inoculation with Rhizoctonia was not a factor. On the other hand, the presence of either soil amendment apparently interfered substantially with the pathogenic action of Rhizoctonia inoculum applied in a band, approximately 2 inches from the beet row, 3 weeks after planting.

Sterilized barley- and sorghum-grain substrates resulted in severe losses in seedling stand, in the field, when applied with the seed at time of planting. On the basis of results obtained from a laboratory experiment, this occurrence, at least with respect to barley, was attributed to stimulation of the pathogenic activity of *Pythium* sp. occurring naturally in the soil.

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Use of an Annual Tester for the Monogerm Conversion of Inbred Lines of Sugar Beets

R. K. OLDEMEYER[1]

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The use of monogerm sugar beet varieties by farmers for reduction of spring labor makes it imperative that all varieties, hybrid or otherwise, be monogerm. The breeding program of The Great Western Sugar Company is directed toward the eventual introduction of a male-sterile hybrid of inbred lines. If the hybrid is a 4-way cross, two of the parental inbred lines will have to be monogerm and "O" type (a genotype which in male-sterile cytoplasm causes male sterility).

The inbred lines presently in the stock of Great Western from which combining ability data has been obtained and which are considered uniform enough for hybrid production are all multigerm. Only in the last two or three years has monogerm material of proven value been available for initiating inbred lines.

In anticipating hybrid variety production, all inbred lines that have high general combining ability are indexed for "O" type. A monogerm conversion is initiated of the lines which are "O" type or are segregating "O" type. A few years saved in converting lines to monogerm will result in substantial profit to the grower.

F. V. Owen (2)[2] suggested using an annual cytoplasmic male-sterile tester to speed identifying "O" type plants. The annual which Owen developed for this purpose is in general use by beet breeders.

The identification of plants heterozygous for the recessive monogerm character is practically impossible in the early backcross generations of inbred lines even though plants heterozygous for monogerm have a reduced locule number; much genetic variation in locule number exists because of modifier genes. If heterozygous plants could be identified, backcrossing to multigerm inbred lines could be continued without a segregating generation for the production of monogerm plants upon which to backcross. For identifying monogerm heterozygotes, an annual monogerm strain, segregating for genetic male sterility, was obtained from F. V. Owen, USDA, ARS, Logan, Utah.

In the diagram, a method is outlined for using the annual monogerm tester in the monogerm conversion of multigerm in-

[1] Director, Seed Development, The Great Western Sugar Company, Agricultural Experiment Station, Longmont, Colorado.

[2] Numbers in parentheses refer to literature cited.

bred lines. The method involves crossing plants of unknown genotypes of the first backcross generation (F_1B_1) to a male-sterile annual monogerm plant. Each corresponding F_1B_1 plant, which has red hypocotyl, is also bagged with a green hypocotyl parental plant or to a parental plant which has been male-sterilized with a selective gametocide, FW450 (1) or FW 676, if the inbred line is homozygous red hypocotyl (With all green hypocotyl and heterozygous hypocotyl color lines, the red hypocotyl gene is used as a cross marker).

The next backcross generation is planted and vernalization is initiated. At the same time the annual test-cross seed is planted and readings for monogerm plants in the progeny are made in about eight weeks without vernalization. Subsequent backcross generations are made involving plants of progenies from parental plants having the monogerm gene as identified by the tester cross; progenies from plants without the monogerm gene are discarded. To extract monogerm plants, a number of backcross plants are intercrossed; without selection and with simple recessive inheritance, one monogerm plant occurs in sixteen plants in the segregating generation.

The value of this method is time saved. Two generations are saved (6 vs. 8) in converting to the third backcross generation, F_1B_3, and four generations to the F_1B_5, 8 vs. 12. One year is saved to the F_1B_3 and two years to the F_1B_5 if two generations are produced per year.

In backcross generations beyond the F_1B_2, it has been possible to identify a high percentage of heterozygotes by phenotype. The number of crosses necessary to assure using heterozygous monogerm plants as parents after the F_1B_2 can be reduced by roguing the highly multiple plants. With some lines, the test cross is not necessary for identifying heterozygotes in later generations if roguing for reduced locule number is carried out.

Backcrossing without extracting the monogerm character has been successful in the conversion of 17 of 18 lines. One line evidently contains a gene or genes of major effect which obscure the monogerm character when the primary monogerm gene is homozygous recessive. A more complicated technique of test crossing and backcrossing might allow continuous backcrossing of this line but the simpler, although slower technique, is the production of a segregating generation and the extraction of the monogerm character in each backcross generation.

The use of a tester line which is a cytoplasmic male sterile would allow control of "O" type during backcrossing. Progress is being made toward the development of such a strain.

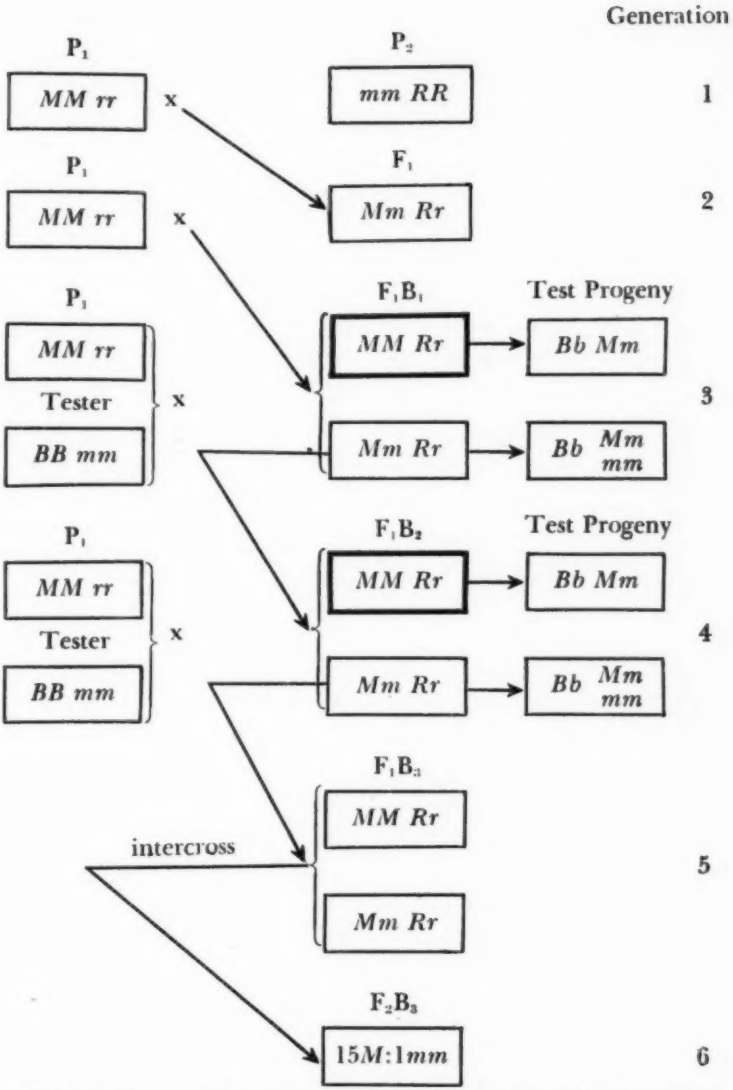

Fig. 1.—Diagram of monogerm backcross conversion of beets using an annual tester to identify the heterozygotes assuming simple recessive inheritance. *M* vs. *m*, multigerm and monogerm genes; *R* vs. *r*, red vs. green hypocotyl; *B* vs. *b*, annual vs. biennial. Encircling with ——— indicates discarding of all backcross progeny from plants not having the *m* gene.

Inbred lines of Great Western are for the most part self-incompatible, having been developed under environmental conditions conducive to selfing. The true nature of the incompatibility system in beets is questionable; as yet no particular trouble has been experienced in seed set by continuous backcrossing although many of the parental lines are probably homozygous for incompatibility alleles as judged from their sib-incompatibility. Perhaps additional evidence will be forthcoming as to the nature of self-incompatibility in beets as the result of continuous backcrossing.

In several instances in converting "O" type lines, most all backcross hybrid progenies have been male sterile when the non-recurrent parent was used as the female of the first cross and the backcross plant rather than the recurrent parent was the female in subsequent backcrosses. It is assumed that an "O" genotype was introduced into male-sterile cytoplasm which originally was not expressed because of the presence of a restorer genotype. It is, therefore, particularly important that either the recurrent parent be used as the female parent of the original cross or the non-recurrent parent be "O" type.

Summary

A tester plant which is annual, monogerm and male sterile can be used to identify heterozygous monogerm plants so that continuous backcrossings can be made in monogerm conversion without losing the monogerm gene.

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Variability of Sugar Beet Plants Grown in Pots Without Competition for Light, Water and Nutrients

ALBERT ULRICH[1]

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Individual sugar beet plants of an open-pollinated variety may differ greatly from one another in size and sugar content when grown side by side in the field and in the greenhouse (3, 7)[2]. The great variability in plants is particularly noticeable down the row just prior to harvest. Neighboring plants may differ in root size by a factor of two and at times by as much as ten or more (3). Depending upon the antecedent conditions this variability in size and in sugar content may often be correlated with such factors as disease, insects or plant spacing. Closely spaced plants will be small because of competition for light (8), nutrients or water; whereas widely spaced plants, because of less competition, will be much larger in size and much lower in sucrose concentration, but the plants growing in any one spacing will still differ considerably from one another.

In addition to the variability associated primarily with environmental factors, there is also the variabilty due to genetic composition. In widely spaced plants much of the variability from plant to plant for open-pollinated varieties is undoubtedly of genetic origin (4, 5) and this determines to a large extent the size and chemical composition of the beet plant at harvest. For closely spaced plants the major source of variation may also be genetic rather than environmental. Here, a young seedling with excellent genetic composition will grow faster at the start; the roots will absorb more nutrients, the tops will produce more leaves and absorb more light energy, and once this plant has taken the lead it will stay ahead in size and crowd out its neighbors.

In contrast to this situation due to genetic dominance, a beet seedling of identical genetic composition may be located by chance on a more favorable spot, and because of better nutrition, aeration and less disease can forge ahead of its neighbors. Because of this interplay of environmental and genetic factors operating in the field, their effects cannot be readily separated from one another as long as beet plants are closely spaced. However, a fairly good estimate of genetic variability can be obtained

[1] Plant Physiologist, Department of Soils and Plant Nutrition, University of California, Berkeley 4, California.

[2] Numbers in parentheses refer to literature cited.

from widely spaced plants (4, 5) in fertile fields or from plants grown outdoors singly in pots without competition for light, water and nutrients. The magnitude of these differences for plants grown in pots without competition is the subject matter of this paper.

Methods and Procedures

The sugar beet plants were grown in 207 pots placed outdoors in full sunlight on a concrete slab at Berkeley, California. All conditions and cultural practices were kept the same so that the effects of environmental variation were at a minimum and the opportunities for genetic expression were at a maximum. The four gallon pots used for the study were painted on the outside with aluminum paint and on the inside with Amercoat Number 33. Drainage was provided for by four holes drilled near the outer edge at the bottom of the pot. Vermiculite number 2 was added a teacup full at a time in rotation until all pots were filled simultaneously. The vermiculite was packed firmly by adding nutrient solution until drainage occurred, followed by dropping each pot five times on the concrete slab. More vermiculite was added to fill the pots to the proper level. Seed of the variety US 75 was planted on May 18, 1954, after treatment with Phygon XL at the rate of 1% by weight. Ten seed balls were planted to a depth of 2 cm at the center of the pot in a circle 9 cm in diameter.

The seeds, seedlings and plants were watered daily with ½ strength modified Hoagland's nutrient solution (9). At each watering sufficient solution was added to allow for some drainage through the holes. During germination and final thinning, the pots were placed in contact with each other in groups of 24 pots, three columns of eight pots each. After the final thinning the pots were separated by a minimum distance of 20 inches on the concrete slab. This spacing was sufficient to prevent an overlap of foliage from neighboring plants for the entire growth period.

Thinning of plants to one plant per pot was done gradually. On June 7, at the 2- to early 3-leaf stage, the plants were thinned to 1 seedling per seedball and 6 seedlings per pot. The subsequent thinnings were on June 11, 15, 17, 21 and 22, and on these dates the plants were approximately in the 4-, 6-, 8-, 10- and 11-leaf stages of growth, respectively. After the final thinning a single plant, uniform in size and development, remained near the center of each pot. Old leaves, less than 50% functional, were removed biweekly and included in the total dry weight of leaves

produced. Spraying with Ortho-mite 15W[3] and Isotox garden spray M[3]. at regular intervals kept red spider and aphid infestations, respectively, under control.

The sugar beet plants were inspected for curly top, virus yellows, mosaic and "red-spot" diseases a few days before the plants were harvested. At the time of harvest on October 26, 1954, the height of the tallest recently matured leaf for each plant was measured and five recently matured leaves were taken per plant and separated into petioles and blades. The remaining leaves on each plant were "shaved off," leaving only the meristematic and axillary buds, which made it possible to produce seed the following year from the roots selected as mother beets. All fresh material was weighed immediately and then dried in a forced draft oven at 80° C. After the dry material was weighed, the petioles and blades were ground in an intermediate Wiley mill to pass a 40 mesh sieve and analyzed for NO_3-N, PO_4-P, K and Na (2).

The beet roots were washed free of vermiculite, freed of fibrous roots and wiped free of moisture before weighing. Beet root weights included root and crown tissues. Fresh beet pulp samples for sucrose determination contained root tissue only and were obtained by removing a V-shaped wedge of root tissue with a Kiel rasp. Three 26.0 gram samples of pulp for a root were weighed, placed individually in 4″ x 2″ x 4″ polyethylene bags, sealed with a heat sealer, and frozen immediately by contact with dry ice (10). The frozen samples were transferred to a deep freeze cabinet maintained at −19° C and analyzed later for sucrose by the hot digestion method (1). A fourth 26.0 gram sample of pulp was dried at 80° C, weighed and ground for chemical analysis.

Results

A detailed inspection of the sugar beet plants before harvest revealed that 14 of 207 plants had a "red-spot" disease of genetic origin and 193 plants were without the "red-spot" disease. Of the 193 plants, 78 were classified as normal, 55 as mildly affected by virus yellows, 10 as mildly affected by sugar beet mosaic, 14 as "severely" affected by sugar beet mosaic and 8 by a combination of virus yellows and sugar beet mosaic. None of the diseases, in spite of their presence, had a significant effect on top growth, beet root size, sucrose concentration or on amount of sugar produced (Table 1). Because of the mildness of the diseases, the 193 beet plants were considered as being "normal" for these

[3] California Spray-Chemical Corporation.

Table 1.—Effect of disease on sucrose concentration, weight of roots, tops and sugar for single plants per pot.

Disease	Number of plants	Beet Root grams	Sucrose %	Sucrose grams	Living Tops Fresh grams	Living Tops Dry grams	Tops total dry matter grams
None	78	2815	13.4	377	997	133	202
Yellows, mild	55	2840	13.3	378	1000	133	204
Mosaic, mild	10	2812	13.6	382	986	134	196
Mosaic, mild recent	28	2758	13.7	380	922	125	193
Mosaic, severe	14	2741	13.5	370	883	121	181
Yellows and mosaic	8	2704	13.5	362	843	117	197
Overall mean	193	2804	13.4	377	972	131	199
F-value[1]	.....	0.46	0.62	0.20	1.15	0.75	1.14
Standard deviation, (s)	.....	347.6	1.14	55.0	266	34.9	38.0
C.V.[2]	.....	12.4	8.4	14.6	27.4	26.7	19.1
Red spot, mild	6	2585	13.3	342	814	114	195
Red spot, severe	8	2001	12.3	246	821	111	191
Red spot, mild and severe	14	2252	12.7	288	817	112	193
F-value[3]	.....	27.0	3.62	26.8	4.73	0.30	0.55

[1] Disease variance (normal plus all diseases exclusive of red spot) divided by error variance. Required F-value at 5% and 1% levels of significance are 2.26 and 3.11.

[2] Coefficient of variability.

[3] Disease variance (normal plus red spot) divided by error variance. Required F-values at the 5% and 1% levels of significance are 3.95 and 6.92.

measurements and for the mineral content of the pulp and of the petioles and blades of the recently matured leaves. Since the "red-spot" disease caused a distinct decrease in beet root weight, top weight, sucrose concentration and in sugar produced (Table 1), these plants were not included in the variability study proper as summarized in Table 2.

The beet root weights for the "normal" plants ranged from 1,607 to 3,667 grams per plant, with a mean value of 2,804, and a coefficient of variation of 12.3 (Table 2). This compares with a range of 129 to 2,315 grams per beet for consecutive plants down the row for the US 33 variety at two locations in a field near Woodland, California (Table 3), and 104 to 2,134 grams per beet for consecutive plants harvested within a row for the Am. #5 variety near the coastal area of Los Angeles County (Table 4). The corresponding coefficients of variability for root weight ranged from 60 to 65 in the two fields. If these variations are typical of field grown beets of open-pollinated varieties such as US 33 and Am. #5, then beet root variability in the field is much greater than for beets grown singly in pots. The coefficient

of variability of 12.3 for the pot grown plants also compares favorably to a value of 27.9 calculated from the data of Powers et al. (5) (see their Tables 4 and 10) for the F_1 hybrid grown noncompetitively in the field and averaging 1.88 lb (853 grams).

The sucrose concentrations of the 193 beets (Table 2) ranged from 10.5 to values as high as 16.0 percent, even though the plants were high in nutrients, including nitrogen. In the field near Los Angeles (Table 4) under a nitrogen and phosphorus deficiency condition, the values ranged from 14.4 to an exceedingly high value of 23.6 percent. The coefficients of variation

Table 2.—Mean, range, standard deviation, coefficient of variability and required number of replications for measurements made on sugar beet plants grown outdoors in pots without competition.

Measurement	Mean	Range	s[1]	CV[2]	Number needed for 10% difference P = 0.05
Beet (g)	2804	1607-3667	345.1	12.3	13
Sucrose (%)	13.4	10.5-16.0	1.13	8.4	7
Sucrose (g)	377	207-507	54.4	14.4	17
Fresh tops (g)	972	239-1803	266.1	27.4	60
Fresh blades (g)	407	113-703	106.6	26.2	50
Fresh residue (g)	557	125-1149	176.0	31.6	70+
Dry tops (g)	130.5	37-223	34.8	26.6	55
Dry blades (g)	63.8	19-109	16.2	25.3	49
Dry residue (g)	66.7	18-132	20.7	31.1	70+
Old leaves (g)	68.5	23-116	19.7	28.8	65
Dry tops + old leaves (g)	199.1	87-314	37.9	19.1	28
Dry pulp (%)	20.4	16.8-24.3	1.31	6.4	6
Leaf count	220.8	60-430	68.2	30.9	70
Height (cm)	27.5	12-40	4.1	14.9	19
NO_3-N (ppm) dry petioles	5552	1530-11,000	1967	35.4	70+
NO_3-N (ppm) dry pulp	1377	530-3040	455	33.0	70+
PO_4-P (ppm) dry petioles	2276	1020-3740	591	26.0	53
PO_4-P (ppm) dry pulp	1651	950-2370	251	15.2	19
K (%) petioles	5.76	3.36-9.18	1.00	17.3	23
K (%) blades	6.05	3.71-9.65	0.843	13.9	17
K (%) dry pulp	1.62	0.74-2.66	0.305	18.9	28
K (%) dry pulp (sugar free basis)	1.87	0.89-2.97	0.329	17.6	24

[1] s, the standard deviation, is the square root of the variance for an individual plant.
[2] CV is the coefficient of variability.

for percent sucrose for the pot and field experiment were 8.4 and 7.5, respectively. These values for variability are somewhat higher than the 5.4 value for the F_1 hybrid of Powers et al. (5) but are much lower than those for the field near Woodland (Table 3), where some plants were deficient and some nondeficient in nitrogen. Here the sucrose concentrations ranged from 4.4 to 17.2 percent and the coefficients of variation from 20.9 to 21.9 percent. The ranges and coefficients of variation for the amount of sugar per beet root are appreciably higher than for the corre-

Table 3.—Variability of sugar beets growing consecutively within the row at two locations near Woodland, California, 1948.

Measurement	Location	Mean[1]	Range	s[2]	C.V.[3]
Sucrose, %	I	12.7	4.4-17.2	2.65	20.9
	II	11.8	6.3-15.7	2.55	21.6
Beets, grams	I	568	129-1816	339	59.7
	II	699	140-2315	452	64.7
Sucrose, grams	I	74.7	11-249	50.9	68.1
	II	84.4	11-306	59.9	71.0
NO_3-N, ppm	I	1950	320-9000	1768	90.7
	II	2404	170-6200	1984	82.5
PO_4-P, ppm	I	1430	410-5100	943	65.9
	II	1202	430-2750	572	47.6
K, %	I	3.88	1.97-6.08	1.12	28.9
	II	4.76	1.13-7.38	1.35	28.4

[1] Mean of 50 consecutive plants at each location.
[2] s, the standard deviation, is the square root of the variance for an individual plant.
[3] C.V. is the coefficient of variability.

Table 4.—Variability of sugar beets growing consecutively within the row at same location near Dominguez, Californa, (August 21, 1950).

Measurement	N	Mean	Range	s[1]	C.V.[2]
Sucrose, %	1-48	18.5	14.4-21.1	1.38	7.5
	49-96	18.6	15.0-23.6	1.40	7.5
Beets, grams	1-48	590	104-1740	373	63.1
	49-96	591	112-2134	355	60.0
Sucrose, grams	1-48	106	20-260	61.9	58.4
	49-96	109	20-320	65.0	59.6
NO_3-N, ppm	1-48	511	70-2030	481	94.1
	49-96	581	60-2250	556	95.7
PO_4-P, ppm	1-48	512	340-730	90.1	17.6
	49-96	509	330-750	94.9	18.6
K, %	1-48	5.43	2.50-8.50	1.14	21.0
	49-96	6.22	2.40-8.72	1.35	21.7

[1] s, the standard deviation, is the square root of the variance for an individual plant.
[2] C.V. is the coefficient of variability.

sponding values for sucrose concentration and slightly higher than for beet-root weight since the amount of sugar per beet is calculated from the product of these values.

A further indication of the variability of sugar beet plants in pots (Table 2) is afforded by the results for top growth, leaf count, plant height, percent dry weight of pulp and for the mineral content of the petioles, blades and pulp. Tops varied in fresh weight from 239 to 1803 grams and in total dry matter from 87 to 314 grams per plant. Coefficients of variation for fresh and dry weight of tops were less than for any of its parts, except for the fresh and dry weight of the leaf blades. An exceptionally low coefficient of variability was registered for percent dry weight of pulp and an intermediate value for plant height. The greatest variability was observed for the fresh and dry weights of the residue material and for the leaf count. The coefficients of variability for the mineral content of the pulp ranged from 15.2 percent for phosphate-phosphorus to 33.0 percent for nitrate-nitrogen. The coefficients of variability for the petioles and blades were the lowest for potassium and the highest for nitrate-nitrogen.

These differences in variability reflect, in part, the precision of the analytical method used and the variability of the constituent within the plant part itself. The greater source of variability is quite likely associated with the plant because the analytical error is so much smaller than the variation among petioles, blades, or pulp. In dry pulp the lowest to highest nitrate value differed by a factor of nearly six fold and for the petioles by approximately seven fold, whereas the corresponding values for potassium are about three fold for pulp, petioles and blades. The relative range of values for phosphate-phosphorus is about the same as for potassium. For field grown beets (Tables 3 and 4), the variability of the petioles in nitrate and potassium, and at times, for phosphate, is greater than the variability for the corresponding values of plants in pots (Table 2). The range of values is also, as a rule, greater for the field than for the pot grown beets.

Discussion of Results

The experimental results for the open-pollinated sugar beet variety, US 75, have shown that there is still a high degree of variability among individual plants in sugar content, size and mineral constituents even when the plants have been cultured carefully in pots by a standardized technique that eliminates completely any competition for light, water, and nutrients. The

variability, however, is much less than that found under field conditions or normally found in greenhouse experiments. In order to detect a difference of 10% between two means at a probability level of 0.05, the required numbers of beets are 9, 31 and 70 for coefficients of variability of 10, 20 and 30%, respectively (6). To detect a difference of 15 and 20% between means, the numbers of beets for coefficients of variability of 10, 20 and 30% drop to 5, 15 and 31, and 4, 9 and 19, respectively. In the present variability trial only 7 beets are required to detect a difference of 10% in sucrose concentration (one to two percentage units), 13 for beet root weight and 17 for amount of sugar per beet root (Table 2). In actual trial runs with two plants per pot, significant differences among closely related sugar beet varieties have been detected at the 5% and 1% levels of significance for changes in sucrose concentrations of 0.7 and 0.9 percentage units, in root weight of 7.8 and 10.3% and in amount of sucrose per beet root of 8.7 and 11.5%, respectively, with only 11 replications (unpublished results of this laboratory). The number of replications required to detect significant differences in top weight and in mineral constituents of pulp and leaf parts tends to be higher (Table 2).

Variation among individual beet plants in the present uniformity trial, can be separated into at least two main categories, one genetic and the other environmental. The environmental variability associated with disease was nonsignificant (Table 1) and from insects was very minor in nature, leaving experimental and genetic variability as the primary causes of variation among the beet plants. At first glance, one might assume that the bulk of the variation would have been due to the nonuniformity of the genetic material and not to the environment. Tests for positional variability were also nonsignificant, again leaving only genetic variability as the primary cause of variation among individuals. However, it is not completely logical to assume that environmental variability, in this case cultural variability, is nonexistent. A measure of genetic and environmental variability could have been obtained by a comparison of an open-pollinated variety with an equally good F_1 hybrid, as suggested by the recent work of Powers (4) and Powers, et al. (5), but at the time of the test in 1954 a comparable hybrid was not available for experimental use. The use of F_1 hybrids in future physiological studies is a step in the right direction and will lead to a further reduction in experimental variability. The growing of two plants per pot and of analyzing the results statistically on

a pot basis will reduce the pot to pot variability by a factor of 0.71, one divided by the square root of two, or a gain in precision of approximately 41%.

Summary

Sugar beet plants of the open-pollinated variety, US 75, were grown outdoors in vermiculite as single plants per pot without competition for light, water or nutrients. Diseases and pests were kept to a minimum and had no significant effect upon the plants. Variability due to environmental factors was kept to a minimum, and yet the coefficients of variability (mostly genetic) remained quite high. The values were: beet root weight, 12.3%; sucrose concentration, 8.4%; sucrose per beet, 14.4%; petiole nitrate-nitrogen, 35.4%; petiole phosphate-phosphorus, 26.0%; and petiole potassium, 17.3%. Considerably higher values were observed as a rule for the same measurements for field grown beets growing consecutively in the row, e.g., 59.7 to 64.7%; 7.5 to 21.6%; 58.2 to 71.0%; 82.5 to 95.7%; 17.6 to 65.9%; and 21.1 to 28.9%, respectively. In spite of the relatively high coefficients of variability for pot grown sugar beet plants, the numbers of beets required to detect a difference of 10% between means at the 5% level of significance were only 13 for beet root weight, 7 for sucrose percent and 17 for amount of sucrose per beet root. To detect a difference of 10% in nutrient content of the petioles among varieties from 19 to 70 or more beets appeared necessary. F_1 hybrids and two plants per pot were suggested as further means of reducing pot to pot variability for sugar beet plants.

Acknowledgment

The author is indebted to Dr. E. S. Sylvester, Associate Entomologist, University of California, for the disease readings made on the sugar beet plants prior to harvest.

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Radiographing as a Method of Observing Some Seed Characters in Monogerm Sugar Beet Fruits[1]

G. J. HOGABOAM[2]

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A sugar beet fruit is made up of a lignified and suberized mass within which are one to several seeds. Seed examination has been a problem since time-consuming dissection of the fruits has been necessary. The X-ray techniques developed to detect insect infestations in wheat (2,3,4)[3] proved to be an answer to the seed examination problem for many types of research. Wiksten (5) recently reported the use of X-ray to study stratification and seed quality in Scotch pine and Norway spruce.

In 1955, E. F. Patton of the General Electric Company's[4] X-ray department provided a radiogram of 3 lots of sugar beet fruits, two multigerm (many-seeded) and one monogerm (one-seeded). Since sugar beet seeds are oblate spherical and are oriented in various planes within the multigerm fruit, the size and shape of all seeds cannot be determined with a single radiograph. However, Grimm (1) in 1958 by X-ray examination was able to predict rather accurately the subsequent germination of whole and segmented multigerm fruits. By mounting the monogerm sugar beet fruits with the "seed cap" down, the seed of all fruits lie in one plane making possible a study of seed characters in the radiograph (Figure 1). This technique was of value in comparisons of seed and fruit size and their consequent effect on the speed of germination[5].

Seeds from each of 19 plants of a monogerm variety were studied. Since monogerm fruits are approximately oblate spheroid, they were screened through a round-hole screen for fruit diameter and then through a slotted screen for fruit thickness. Categories were made by plant source for fruit diameter and thickness in preparation for radiographing. Since the required X-ray exposure might vary with fruit thickness, fruits with similar thicknesses were mounted on the same template for radiographing. The templates, 13" x 16", were prepared from 1/8" tempered fiberboard by cutting 48 - 1" x 1 3/8" openings. Strips of cellophane tape were placed on the lower side of the template across the

[1] Cooperative investigations of the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, and the Michigan Agricultural Experiment Station. Approved for publication as Journal Article No. 2769, Michigan Agricultural Experiment Station.

[2] Research Agronomist, Crops Research Division, Agricultural Research Service, U. S. Department of Agricultural, East Lansing, Michigan.

[3] Numbers in parentheses refer to literature cited.

[4] Trade and company names are mentioned for identification only and do not constitute recommendation by United States Department of Agriculture.

[5] A manuscript is in preparation by Hogaboam, G. J. and F. W. Snyder on influence of size of fruit and seed on germination of a monogerm sugar beet variety.

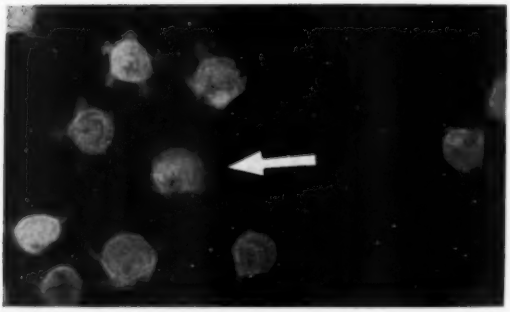

Figure 1.—Portion of the original radiograph of monogerm sugar beet fruits. An empty fruit containing no viable seed may be seen in the lower left corner. A fruit containing two seeds is marked by the arrow. Light areas represent resistance to X-ray penetration, thus the seeds appear light. The ligneous inner layer of the seedballs is outlined as light at the edges where the layer is vertical. This is especially noticeable with empty seedballs.

openings. The fruits to be radiographed were then mounted in rows on the tape in these openings. As a check on distortion and as a standard for measurement, size gauges were placed in three random positions on each template.

Because a very soft X-ray is needed to differentiate the seed within the sugar beet fruit and show details of seed structure, General Electric's low-voltage X-ray unit, model LC-90,[4] was used. Very good results[6] were obtained on 14″ x 17″ Industrial type M film using 3 and 5 milliamperes, 15 and 25 K.V.P., with exposures varying from 1½ to 2½ minutes, with a 30″ focal film distance.

The template holding the fruits was placed in contact with the film for radiographing. The duration of radiographic exposure depended upon the detail desired. Within limits, the greater the duration the greater the detail within the seed in the radiograph. Since the outer portions of the sugar beet fruit are suberized and the inner portions surrounding the seed lignified, a shorter exposure would show the suberized material of the fruit at the expense of seed detail.

The true-seed diameter (Figure 2) can be measured either by projection of the radiograph or by a direct measurement of the contact film. When the projection method is used, a size gauge must also be radiographed and projected to give a basis for measurement. Direct measurement of the radiographed size gauges revealed very little distortion. Since dark film from long exposure does not project well, a relatively short exposure must

[6] The author is indebted to J. Heck, D. Rognlie, and R. Brooks of the X-ray Department, General Electric Company[6] for contributing their time and skill in making the radiographs used in this study.

be used at the expense of some seed detail (Figure 3). Each template opening was cut from the film and mounted in 2" x 2" slides for projection. Projected size gauges and seeds were then compared.

The direct measurement method is preferred since darker radiograms with more detail (Figure 4) can be used. With this method, a mirror is used to reflect bright light up through the radiograph and the seed diameter is measured directly with a size gauge.

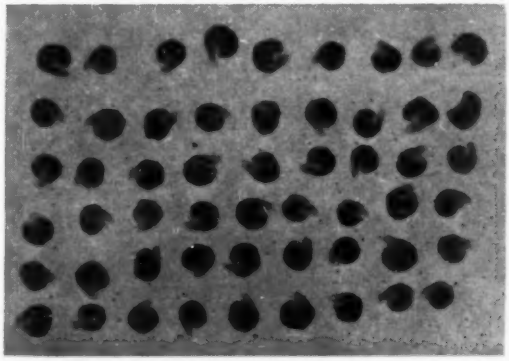

Figure 2.—Radiograph of excised true seeds of sugar beets showing differences in shape. Negative reproduction.

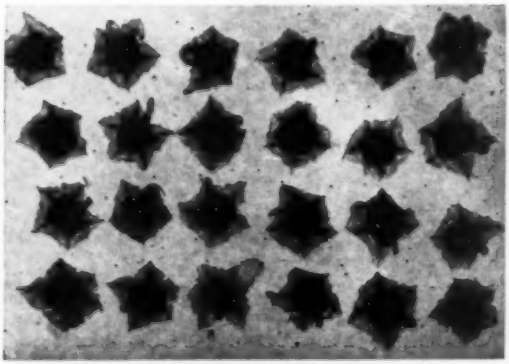

Figure 3.—Radiograph of monogerm sugar beet fruits made with an X-ray exposure of 1½ minutes at 3 M.A. and 15 K.V.P. A film with this exposure is suitable for projection. More details of the fruit are visible but details within the seed cavity are difficult to observe. Negative reproduction.

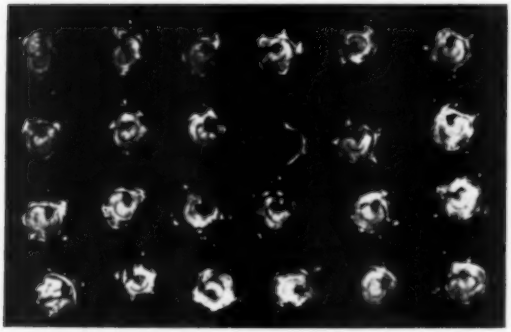

Figure 4.—Radiograph of monogerm sugar beet fruits made with an X-ray exposure of 2 minutes at 5 M.A. and 25 K.V.P. Such a radiograph is suitable for direct observation over a bright light. Only the most lignified portions of the fruit are visible but details of the seed are clearly visible. Note the empty fruit in the third row from the bottom, fourth from the left. Fruits containing two seeds are located on the left side in the top and bottom rows. The third and fifth fruit from the left in the third row from the bottom contain bump-like growths in addition to a viable seed within the cavity.

The germination performance and seedling appearance indicated that the exposure of the dry seeds to the roentgen level employed had no perceptible deleterious effects.

Some of the observations follow:

1. Seed from each plant source examined contained some seedless fruits. One lot had as few as 2% seedless fruits whereas another had as many as 35%.
2. Sixteen of the 19 monogerm plants had some fruits which contained 2, 3, or 4 seeds in the seed cavity. The percentages of such fruits varied from 0.3 to 23.8.
3. A few twin embryos were found.
4. Occasional bump-like growths occurred in the seed cavity along with a well-developed seed (Figure 4).
5. The shape of the seed varied from oblong to round, when viewed from the top (Figure 2).
6. The perisperm of some seeds was not fully developed although the embryo had developed normally.
7. One seed cavity contained a fully developed perisperm but an underdeveloped embryo.
8. When mounting the fruits on the templates, striking differences between plants were noted in the size of the openings (vascular trace scars) into the seed cavity at the point of attachment of the fruit.

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Some Chemical-Genetic Studies Pertaining to Quality in Sugar Beets (*Beta vulgaris* L.) [1, 4]

MERLE G. PAYNE[2], LEROY POWERS[3], AND E. E. REMMENGA[2]

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Quality of the sugar beets delivered to the factories for processing has become of considerable concern in many sugar-beet producing areas of the United States. In some areas the percentage sucrose is so low that it is questionable whether factories can continue to operate at a profit. It is thought that the application of increased amounts of nitrogenous fertilizers may be responsible at least in part for the decrease noted in percentage sucrose. The purpose of this article is to report the results from some chemical-genetic studies pertaining to quality in sugar beets (*Beta vulgaris* L.). Particular emphasis will be placed on the interrelations of percentage sucrose, total nitrogen, betaine and glutamic acid.

Experimental Considerations

The design of the experiment is depicted by the tabulations listed in Table 1. There are two treatments, fertilized and non-fertilized. The fertilized plots received a surface application of 100 pounds of available nitrogen (N) and 250 pounds of available phosphorus (P_2O_5) per acre on April 4, 1956. The fertilizer was cultivated under with a rototiller. The experiment was planted on April 10 and 11. On June 26, another 100 pounds of N per acre were drilled in the center of each space between rows of the fertilized plots. The fertilizer treatments are split blocks of replications and are randomized within each replication. The six populations are randomized within each treatment. The locations refer to the position of the eight plants harvested from each plot. For more details about the experimental design see (7) [5].

[1] Cooperative investigations of the Colorado Agricultural Experiment Station the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, and the Beet Sugar Development Foundation. The Colorado State University gratefully acknowledges financial support from the James G. Boswell Foundation administered by the Agricultural Research Center of Stanford Research Institute, the National Institute of Health, and the Beet Sugar Development Foundation and contract-research-funds from the Agricultural Research Service of the U. S. Department of Agriculture. These studies would not have been possible if these funds had not been granted. Approved by the Colorado Agricultural Experiment Station for publication as Scientific Series Article No. SS718.

[2] Associate Professor of Chemistry and Associate Professor of Statistics, respectively, Colorado State University.

[3] Geneticist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture.

[4] The writers are indebted to The Great Western Sugar Company for obtaining thin juice samples by an oxalate method standard with that company and for the percentage apparent purity determinations. These thin juice samples and the apparent purity determinations were obtained from R. R. Wood of that company. Also the writers acknowledge the cooperation of D. W. Robertson, Robert S. Whitney, and W. R. Schmehl in conducting the field experiment. The writers are indebted to the Western Data Processing Center at the University of California at Los Angeles for use of the computing facilities for analyzing data, job No. 398.

[5] Numbers in parentheses refer to literature cited.

Table 1.—Sources of variation designated as main effects, and number of each.

Main effects	Number
Replications	40
Populations	6
Treatments	2
Locations	8

The materials used in the study are populations and fertilizer treatments. The populations are A54-1, A54-1BB, 50-406BB, 50-406, F_1 hybrid, and 52-307. A54-1 is a commercial variety and A54-1BB resulted from seed harvested from 25 mother beets of A54-1. The 25 mother beets giving rise to population A54-1BB were grown in an isolated seed plot along with 25 mother beets from each of 22 other populations. This isolation plot was composed of 25 rows in each of which one mother beet from each population occurred at random, making a total of 23 mother beets per row. Seed was harvested from all mother beets of A54-1 on an individual plant basis and then was bulked to provide population A54-1BB. Seed saved from mother beets of 50-406 and handled in a similar manner produced the population designated as 50-406BB. Hence, since 50-406 is an inbred, 50-406BB is a topcross. Populations 50-406 and 52-307 are inbreds and the F_1 hybrid population resulted from crossing them.

The methods used in analyzing the data have been reported in previous articles (4, 5, 6, 7). All the analyses for total nitrogen, betaine and glutamic acid are from thin juice samples. These three characters are reported as milligrams per 100 milliliters of thin juice equated to a refractometer reading of 10. The method of determining percentage of sucrose is standard with the sugar companies and is well known. The data for glutamic acid were transformed to logarithms for calculating variances, covariances, regressions and correlation coefficients. However, the means for glutamic acid as presented in the tables are calculated from the data on the arithmetic scale.

The statistical constants employed in studying the data are means, variances, covariances, regressions and correlation coefficients.

Both the environmental and genetic variabilities are subject to a certain amount of control. The environmental variability may be controlled to some extent by cultural and fertilizer practices and the genetic variability by breeding varieties and hybrids giving the desired response to these cultural and fertilizer practices. The purpose of this article is to report the results from

studies of the environmental and genetic variances in an attempt to learn to what extent the environment and genotype can be molded to suit the requirements of the beet sugar industry and to learn what procedures to follow to accomplish the desired results.

Method of Chemical Analysis

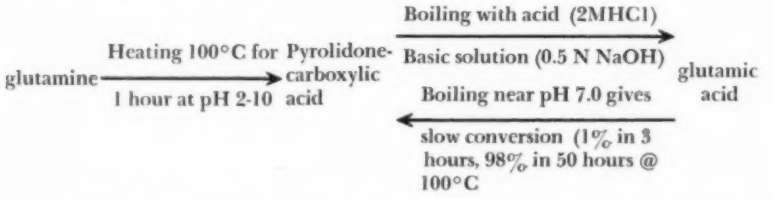
The method of determining total nitrogen was reported by Payne et al., see (4).

Betaine

Quantitative determinations of betaine (trimethylglycine) in thin beet juice samples were made by a procedure described by Focht, Schmidt and Dowling (1). Aliquots of 5 ml of thin beet juice samples were transferred at room temperature in 30 ml beakers. The beakers were placed in the refrigerator at 3°C for about 30 minutes. By experimentation, it was found that adjustment of the sample to a pH 1.0 was not necessary, and since the thin beet juice samples were not discolored, they were not filtered through carbon. The samples were carried through the procedure outlined by Focht et al., with the above changes. The milligrams of betaine per ml of sample were determined by comparing the readings obtained on the Beckman spectrophotometer to the readings on a standard curve.

Glutamic Acid

The determination of glutamic acid content of thin juice was indirectly the determination of glutamine in the original sugar beet. During the oxalation process of preparing the thin juice, some of the glutamine is converted to pyrolidonecarboxylic acid (PCA) and some to glutamic acid, Wilson and Cannan (13).


Boiling with acid (2MHCl)
Heating 100°C for
1 hour at pH 2-10
Pyrolidone-carboxylic acid
Basic solution (0.5 N NaOH)
Boiling near pH 7.0 gives
glutamic acid
glutamine
slow conversion (1% in 3 hours, 98% in 50 hours @ 100°C

Since it is more difficult to determine total glutamine and PCA content than glutamic acid, we chose to hydrolyze the existing glutamine and PCA to glutamic acid and determine the total glutamic acid.

Several methods of determining glutamic acid (2, 3, 9, 10) in thin juice samples were investigated. A chromatographic method

was chosen. Several different hydrolytic procedures were tried on the thin juice samples (8, 12, 13). In each procedure, the hydrolyzing mixture was heated in a water bath for different time intervals at boiling temperature (94°C).

Hydrolysis with 2-4 N HCl caused the formation of a brown precipitate and a brown-colored solution which interfered with spotting for chomatographic study.

Hydrolysis in a very basic solution showed good conversion to glutamic acid, but the high alkaline salt content caused tailing and irregularly shaped spots on the chromatogram.

Alkaline hydrolysis in 0.5 N NaOH heated for 6 hours in the water bath showed complete conversion to glutamic acid on all samples except those with very high glutamine. The PCA conversion was about 98% complete at this time. The hydrolysate was satisfactory for chromatographic purposes.

Method

One ml of the thin juice sample was transferred into a graduated tube (15 ml centrifuge tube), and 0.1 ml of 5 N NaOH was added and the mixture was heated in a water bath at boiling temperature (94°C) for 6 hours. The water level of the bath was maintained so that the digestion mixture in the tube was always completely immersed. Also during the digestion a few drops of distilled water were added at intervals to maintain a volume of approximately 1.0 ml. After 6 hours, the samples were removed, cooled to room temperature, and made up to a volume of 2.0 ml with distilled water for spotting.

For one dimensional chromatograms, 5 microliters of the diluted hydrolyzed sample were placed on Whatman No. 1 filter paper (20 cm x 20 cm). The solvent used was 80 percent phenol (Merck-reagent grade). The chromatographing was continued until the solvent nearly reached the top of the paper (about 6 hours). The papers were then removed, hung in a hood[6] to dry over night at room temperature, then dipped in 0.3 percent ninhydrin in 95 percent ethyl alcohol to reveal the spots, and dried again, away from sunlight.

After 18 hours, the dried, stained papers were cut and passed through the Spinco Analytrol which automatically measures the density of the spot and records the measurement in square centimeters. The concentration of glutamic acid in mg per ml was determined by comparison of the sample readings to a standard curve made from samples of known concentration.

[6] Contamination by some fumes may cause discoloration to the stained papers later, if care is not taken while chromatographs dry.

Comparison of Warburg and Chromatographic Methods for Total Glutamic Acid Determination

To check the accuracy of the chromatographic method for determination of glutamic acid quantitatively, eight thin juice samples, two known samples of glutamine and glutamic acid, and two thin juice samples plus known amounts of glutamine and glutamic acid were hydrolyzed and analyzed by the Warburg method (11) and by the chromatographic method. The comparative results are shown below:

Sample no.	Content	I Warburg (mg per ml)	II Chromatograph (mg per ml)	Deviation of method II from method I in (mg per ml)	Percent deviation
1	Glutamine 1 mg/ml	0.965 mg/ml	0.98 mg/ml	+0.015	1.6
2	Glutamic Acid 1 mg/ml	0.965	0.99	+0.025	2.6
3	Thin Juice	0.43	0.48	+0.05	11.6
4	Thin Juice	0.885	0.96	+0.075	8.5
5	Thin Juice	0.685	0.76	+0.075	11.0
6	Thin Juice	0.815	0.83	+0.015	1.8
7	Thin Juice	0.49	0.52	+0.03	6.1
8	Thin Juice	0.825	0.82	—0.005	0.6
9	Thin Juice	0.715	0.72	+0.005	0.7
10	Thin Juice	0.72	0.70	—0.02	2.8
11	Sample 3+ 0.4 mg/ml Glutamine	0.78	0.81	+0.03	3.8
12	Sample 3 + 0.4 mg/ml Glutamic Acid	0.83	0.88	0.05	6.0
				Average % difference	4.75%

Since the laboratory facilities could not use the Warburg method on 4300 samples, the chromatographic method was used.

Results

An examination of Table 2 reveals that there are differences between populations and fertilizer treatments for percentage sucrose, total nitrogen, betaine and glutamic acid. Also from other data there were found to be marked differences between replications for all of these characters. For example, replication group 33 to 40 was found to be low in percentage sucrose and high in total nitrogen, betaine and glutamic acid. The differences between fertilizer treatments and replications are attributable to environment. The differences between populations contribute primarily to genetic variability. However, there is a negligible amount in this experiment of environmental variability included with the genetic variability due to differences between population.

Each population for each fertilizer treatment is composed of 320 plants. It is apparent that the differences between plants comprise the total variability for populations within fertilizer treatments. In turn this total variability of a given population

Table 2.—The means and their standard errors for percentage sucrose, nitrogen, betaine and glutamic acid, population genetic studies 1956, fertilized and non fertilized.

Populaton and treatment	Sucrose	Nitrogen[1]	Betaine[1]	Glutamic acid[1]
A54-1	%	mg	mg	mg
Fertilized	16.8±0.10	46.8±1.44	116.6±1.53	80.1±3.98
Non-fertilized	17.9±0.10	18.8±0.91	89.8±1.48	12.8±1.87
A54-1BB				
Fertilized	16.7±0.12	44.8±1.42	115.1±1.49	41.4±2.79
Non-fertilized	17.8±0.11	16.9±0.73	85.5±1.62	7.6±0.88
50-406BB				
Fertilized	17.3±0.10	33.6±1.32	106.1±1.33	45.8±3.50
Non-fertilized	17.6±0.09	12.6±0.62	69.9±1.40	6.4±0.93
50-406				
Fertilized	16.1±0.09	31.2±0.82	125.3±1.97	17.3±1.35
Non-fertilized	17.4±0.08	14.6±0.59	73.4±1.55	8.0±0.92
F_1				
Fertilized	17.6±0.09	21.3±0.58	101.3±1.20	18.0±1.18
Non-fertilized	17.6±0.07	9.8±0.31	51.3±1.14	3.5±0.28
52-307				
Fertilized	16.6±0.09	18.6±0.46	108.7±1.22	10.0±0.50
Non-fertilized	16.5±0.10	11.1±0.28	79.8±1.38	3.0±0.14

[1] Milligrams per 100 milliliters of thin juice equated to a refractometer reading of 10.

is composed of two parts, namely, that due to environmental variability within the experimental area and that due to heritable differences between plants. The latter is termed genetic variability and arises from the fact that some plants have different genotypes. The results from the studies within populations and fertilizer treatments will be considered first.

Environmental Variability

The correlation coefficients and the percentages of the variances accounted for by regression are given in Table 3. They are averages derived from the data for the inbreds 50-406, 52-307, and their F_1 hybrid. Hence the correlation coefficients and regressions measure the relations between characters attributable to environmental variability. The data show the relations between percentage sucrose and total nitrogen, betaine and glutamic acid

Table 3.—Correlation coefficients and percentages of the variances accounted for by regression, between plants, average of 50-406, F_1, and 52-307, environmental.[1].

Characters correlated	Correlation coefficient r	% accounted for by regression
Fertilized		
Sucrose vs. nitrogen	—0.48	23.0
Sucrose vs. betaine	—0.09	0.8
Sucrose vs. glutamic acid	—0.28	7.8
Non-fertilized		
Sucrose vs. nitrogen	—0.30	9.0
Sucrose vs. betaine	0.03	0.1
Sucrose vs. glutamic acid	—0.25	6.2

[1] At the 0.05 level $r = 0.06$ and at the 0.01 level $r = 0.08$. These are approximations.

on the fertilized and non-fertilized plots. The relations between sucrose and nitrogen, and sucrose and glutamic acid are negative on both the fertilized and non-fertilized plots. There seems to be very little relation, if any, between sucrose and betaine. The percentages of the variances accounted for by regression are not great, the largest amount being 23.0 percent for sucrose and nitrogen.

The corresponding relations for nitrogen, betaine and glutamic acid are given in Table 4. The relations are all positive and the correlation coefficients are considerably larger than those involving sucrose. The highest correlation coefficients are for glutamic acid and nitrogen and the lowest are for betaine and glutamic acid. The percentages of the variances accounted for by regression range from 16 to 46.2.

Table 4.—Correlation coefficients and percentages of the variances accounted for by regression, between plants, average of 50-406, F_1, and 52-307, environmental.[1]

Characters correlated	Correlation coefficient r	% accounted for by regression
Fertilized		
Betaine vs. nitrogen	0.47	22.1
Glutamic acid vs. nitrogen	0.68	46.2
Betaine vs. Glutamic acid	0.40	16.0
Non-fertilized		
Betaine vs. nitrogen	0.65	42.2
Glutamic acid vs. nitrogen	0.66	43.6
Betaine vs. glutamic acid	0.49	24.0

[1] At the 0.05 level $r = 0.06$ and at the 0.01 level $r = 0.08$. These are approximations.

The conclusions that can be drawn from Tables 3 and 4 are that, as regards individual plants, percentage sucrose is largely independent of total nitrogen, betaine and glutamic acid. This would indicate that there are environmental conditions which allow individual beets to have fair amounts of nitrogen, betaine and glutamic acid and still be fairly high in percentage sucrose. If the agronomist can learn what these environmental conditions are, it may be possible to follow cultural and fertilizer practices that would result in higher percentage sucrose in beets delivered to the factory.

The correlation coefficients for glutamic acid and nitrogen are positive and fairly high. It seems that more difficulty will be involved in controlling the environment so that the commercial crop harvested is low in one of these two characters and high in the other. Manipulation of the environment may lead to low betaine and high glutamic acid or vice versa as only 16.0 percent of the total variance of one on the other is accounted for by regression on the fertilized plots, and only 24.0 percent on the non-fertilized plots.

The correlation coefficients and percentages of the variances accounted for by regression for the two inbreds and the F_1 are given in Table 5. The data are for the fertilized and non-fertilized plots.

For the fertilized plots and for 50-406, sucrose is most closely associated with nitrogen, 32.5 percent of the variance being accounted for by regression. The only statistically significant correlation involving sucrose and betaine is for inbred 50-406. Again the relation is negative. However, only 6.2 percent of the variance is accounted for by regression.

Table 5.—Correlation coefficients and percentages of the variances accounted for by regression, between plants, environmental.[1]

Population and characters correlated	Fertilized		Non-fertilized	
	Correlation coefficient r	% accounted for by regression	Correlation coefficient r	% accounted for by regression
50-406				
Sucrose vs. nitrogen	—0.57	32.5	—0.53	28.1
Sucrose vs. betaine	—0.25	6.2	—0.30	9.0
Sucrose vs. glutamic acid	—0.39	15.2	—0.37	13.7
F_1				
Sucrose vs. nitrogen	—0.54	29.2	—0.36	13.0
Sucrose vs. betaine	—0.01	0.0	—0.04	0.2
Sucrose vs. glutamic acid	—0.31	9.6	—0.37	13.7
52-307				
Sucrose vs. nitrogen	—0.31	9.6	0.03	0.1
Sucrose vs. betaine	0.09	0.8	0.38	14.4
Sucrose vs. glutamic acid	—0.11	1.2	—0.06	0.4

[1] At the 0.05 level $r = 0.11$ and at the 0.01 level $r = 0.15$.

The corresponding data for the non-fertilized plots are given in Table 5 also. For 50-406, sucrose is negatively correlated with nitrogen, betaine and glutamic acid and all the correlation coefficients are significantly different from zero. This is quite a contrast with the correlation coefficients for 52-307. For 52-307 the only statistically significant correlation coefficient is between sucrose and betain and it is positive. Hence all populations do not show the same relations between sucrose and nitrogen, betaine and glutamic acid. Stated genetically there are interactions between genotypes and the environments. However, in no case is more than 28.1 percent of the variance of sucrose accounted for by regression.

The corresponding correlation coefficients showing the closeness of the environmental relations for nitrogen, betaine and glutamic acid on the fertilized plots are given in Table 6. The correlation coefficients are all positive and significantly higher for 52-307 than for 50-406 and the F_1 hybrid. However, that for glutamic acid and nitrogen is not significantly so at the 0.05 level.

Again there are interactions between populations (genotypes) and the environment. For betaine and nitrogen, the percentage of the variance accounted for by regression is more than twice as great for 52-307 than it is for the other two populations. For betaine and glutamic acid, regression accounts for approximately four times as much of the variance in 52-307 as it does in the other two populations.

Table 6.—Correlation coefficients and percentages of the variances accounted for by regression, between plants, environmental.[1]

Population and characters correlated	Fertilized		Non-fertilized	
	Correlation coefficient r	% accounted for by regression	Correlation coefficient r	% accounted for by regression
50-406				
Betaine vs. nitrogen	0.44	19.4	0.73	53.3
Glutamic acid vs. nitrogen	0.65	42.2	0.79	62.4
Betaine vs. glutamic acid	0.32	10.2	0.68	46.2
F_1				
Betaine vs. nitrogen	0.40	16.0	0.58	33.6
Glutamic acid vs. nitrogen	0.71	50.4	0.62	38.4
Betaine vs. glutamic acid	0.34	11.6	0.37	13.7
52-307				
Betaine vs. nitrogen	0.65	42.2	0.59	34.8
Glutamic acid vs. nitrogen	0.74	54.8	0.47	22.1
Betaine vs. glutamic acid	0.65	42.2	0.32	10.2

[1] At the 0.05 level $r = 0.11$ and at the 0.01 level $r = 0.15$.

The corresponding data for the non-fertilized plots are given in Table 6 also. Here the relations are reversed as the higher correlation coefficients are found within population 50-406 as compared with 52-307. The percentage of the variance accounted for by regression is considerably higher for 50-406. Comparing the data for the two fertilizer treatments it can be seen that there is a distinct genotype-environment interaction.

In addition to the data for individual plants within the inbreds and F_1 hybrid, the differences between fertilizer treatments and the differences between replications provide a means of studying the environmental inter-relations between sucrose and nitrogen, betaine and glutamic acid, and between nitrogen, betaine and glutamic acid. The combined data for the fertilized and non-fertilized plots are given in Table 7. The correlation coefficients for sucrose and nitrogen, and sucrose and glutamic acid are extremely high, a little over 80 percent of the environmental variance being accounted for by regression. The relation is negative. Also the relation between glutamic acid and nitrogen is extremely high and positive, 91.5 percent of the environmental variability being accounted for by regression. This means that those cultural and fertilizer practices which tended to increase nitrogen and the

nitrogenous compounds on the average correspondingly decreased sucrose. On the other hand, cultural and fertilizer practices which tended to increase total nitrogen also tended to increase correspondingly betaine and glutamic acid. These data definitely point out the advantages of using sound fertilizer and cultural practices. Much can be accomplished toward increasing the percentage of sucrose in the sugar beets delivered to the factory by so doing.

Table 7.—Correlation coefficients and percentages of the variances accounted for by regression, between replications, environmental.[1]

Characters correlated	Correlation coefficient r	% accounted for by regression
Sucrose vs. nitrogen	—0.91	83.2
Sucrose vs. betaine	—0.70	48.7
Sucrose vs. glutamic acid	—0.90	81.4
Betaine vs. nitrogen	0.88	76.7
Glutamic acid vs. nitrogen	0.96	91.5
Betaine vs. glutamic acid	0.84	70.4

[1] At the 0.05 level r = 0.30 and at the 0.01 level r = 0.39.

Genetic Variability

It has been seen that much can be accomplished by controlling the environment. Next, the bearing the data have on the interrelations between percentage sucrose, total nitrogen, betaine and glutamic acid and between total nitrogen, betaine and glutamic acid will be considered. Again the correlation coefficients and regressions will be studied in connection with the genetic variances to determine these relations.

The data for the individual plants are given in Table 8. These are averages over the three segregating populations. Percentage sucrose is negatively associated with total nitrogen, betaine and glutamic acid on the fertilized plots and with nitrogen on the non-fertilized plots. The associations between sucrose and nitrogen and between sucrose and betaine on the fertilized plots do not differ materially.

Table 8.—Correlation coefficients and percentage of the variances accounted for by regression, between plants average of A54-1, A54-1BB and 50-406BB, genetic.[1]

Characters correlated	Correlation coefficient r	% accounted for by regression
Fertilized		
Sucrose vs. nitrogen	—0.30	9.0
Sucrose vs. betaine	—0.34	11.6
Sucrose vs. glutamic acid	—0.20	4.0
Non-fertilized		
Sucrose vs. nitrogen	—0.32	10.2
Sucrose vs. betaine	0.06	0.4
Sucrose vs. glutamic acid	—0.05	0.2

[1] At the 0.05 level r = 0.06 and at the 0.01 level r = 0.08. These are approximations.

Corresponding data for total nitrogen, betaine and glutamic acid are listed in Table 9. Again these values are over-all averages for the segregating populations. On the fertilized plots the correlation coefficients are positive and highest for betaine and total nitrogen, next highest for glutamic acid and nitrogen and lowest for betaine and glutamic acid. On the non-fertilized plots the correlation coefficients are highest for glutamic acid and nitrogen and lowest for betaine and glutamic acid.

Table 9.—Correlation coefficients and percentage of the variances accounted for by regression, between plants, average of A54-1, A54-1BB and 50-406BB, genetic.[1]

Characters correlated	Correlation coefficient r	% accounted for by regression
Fertilized		
Betaine vs. nitrogen	0.66	43.6
Glutamic acid vs. nitrogen	0.45	20.2
Betaine vs. glutamic acid	0.04	0.2
Non-fertilized		
Betaine vs. nitrogen	0.39	15.2
Glutamic acid vs. nitrogen	0.73	53.3
Betaine vs. glutamic acid	0.30	9.0

[1] At the 0:05 level r = 0.06 and at the 0.01 level r = 0.08. These are approximations.

The data for each of the three segregating populations are listed in Table 10. On the fertilized plots the highest correlation coefficients involving the genetic variances and covariances are for population 50-406BB. They are all negative. The same is true of population A54-1BB, except that the correlation coefficients are not nearly so high. In population A54-1BB the greatest amount of the variance accounted for by regression is 9.0 percent and it is for sucrose and nitrogen. The only significant correlation coefficient for A54-1 is between sucrose and betaine. Clearly, populations differ in the closeness of the relation between sucrose and total nitrogen and between sucrose and the nitrogenous compounds as regards the genetic variability on the fertilized plots. It should be much easier to breed high sucrose at the higher fertility level working with A54-1 than to do so by working with 50-406BB.

The data in Table 10 show that on the non-fertilized plots the relation between sucrose and total nitrogen is negative for all three segregating populations. This is also true of sucrose and betaine and sucrose and glutamic acid for A54-1. The relations between sucrose and these two nitrogenous compounds are positive and significant for A54-1BB. For population 50-406BB the correlation coefficients are not significantly different from zero. Comparing the correlation coefficients for populations and treatments reveals that again there is a decided genotype-environment

Table 10.—Correlation coefficients and percentages of the variances accounted for by regression, between plants, genetic.[1]

Population and characters correlated	Fertilized		Non-fertilized	
	Correlation coefficient r	% accounted for by regression	Correlation coefficient r	% accounted for by regression
A54-1				
Sucrose vs. nitrogen	0.04	0.2	—0.52	27.0
Sucrose vs. betaine	—0.42	17.6	—0.14	2.0
Sucrose vs. glutamic acid	—0.04	0.2	—0.27	7.3
A54-1BB				
Sucrose vs. nitrogen	—0.30	9.0	—0.12	1.4
Sucrose vs. betaine	—0.13	1.7	0.20	4.0
Sucrose vs. glutamic acid	—0.27	7.3	0.30	9.0
50-406BB				
Sucrose vs. nitrogen	—0.63	40.0	—0.32	10.2
Sucrose vs. betaine	—0.81	65.6	0.03	0.1
Sucrose vs. glutamic acid	—0.83	68.9	0.04	0.2

[1] At the 0.05 level r = 0.11 and at the 0.01 level r = 0.15. These are approximations.

interaction. In no case are the percentages accounted for by regression large.

The data showing the genetic relations between total nitrogen, betaine and glutamic acid for the fertilized and non-fertilized plots are listed in Table 11. On the fertilized plots correlation coefficients are positive and highest for 50-406BB. The association is particularly high between total nitrogen and glutamic acid for populations A54-1BB and 50-406BB. In all three segregating populations the correlation coefficients are positive and rather high for total nitrogen and betaine. For populations A54-1 and A54-1BB the correlation coefficients for betaine and glutamic acid are not significantly different from zero.

Table 11.—Correlation coefficients and percentages of the variances accounted for by regression, between plants, genetic.[1]

Population and character correlated	Fertilized		Non-fertilized	
	Correlation coefficient r	% accounted for by regression	Correlation coefficient r	% accounted for by regression
A54-1				
Betaine vs. nitrogen	0.64	41.0	0.69	47.6
Glutamic acid vs. nitrogen	0.23	5.3	0.80	64.0
Betaine vs. glutamic acid	—0.02	0.0	0.63	39.7
A54-1BB				
Betaine vs. nitrogen	0.61	37.2	0.16	2.6
Glutamic acid vs. nitrogen	0.90	81.0	0.64	41.0
Betaine vs. glutamic acid	—0.03	0.1	—0.35	12.2
50-406BB				
Betaine vs. nitrogen	0.86	74.0	0.28	7.8
Glutamic acid vs. nitrogen	1.00	100.0	0.69	47.6
Betaine vs. glutamic acid	0.42	17.6	0.54	29.2

[1] At the 0.05 level r = 0.11 and at the 0.01 level r = 0.15. These are approximations.

The data showing the genetic relations between total nitrogen, betaine and glutamic acid for the non-fertilized plots are given in Table 11 also. The correlation coefficients are higher for A54-1 than they are for 50-406BB. This is the reverse of what was true on the fertilized plots. The values are lowest for A54-1BB, being negative for betaine and glutamic acid. Again there are decided genotype-environment interactions as evidenced by comparing the percentages of the variances accounted for by regression for populations and fertilizer treatments.

Comparisons between the environmental and genetic correlation coefficients and percentages of the variances accounted for by regression between replications and between populations are given in Table 12. The relations between percentage sucrose and total nitrogen, and percentage sucrose and glutamic acid are extremely high for environmental. In both cases better than 80 percent of the environmental variability of percentage sucrose is accounted for by regression. The relation between sucrose and betaine is also negative and fairly close. This means that cultural and fertilizer practices have to be such as not to result in excess amounts of nitrogen and nitrogenous compounds if beets high in percentage sucrose are to be produced. These are environmental influences and are subject to considerable immediate control.

Table 12.—Correlation coefficients and percentages of the variances accounted for by regression between replications and between populations, environmental and genetic.

Character and variability	Correlation coefficient r	% accounted for by regression
Sucrose vs. nitrogen		
Environmental[1]	—0.91	83.2
Genetic[2]	0.28	7.9
Sucrose vs. betaine		
Environmental[1]	—0.70	48.7
Genetic[2]	—0.46	21.3
Sucrose vs. glutamic acid		
Environmental[1]	—0.90	81.4
Genetic[2]	0.50	25.0

[1] At the 0.05 level r = 0.30 and at the 0.01 level r = 0.39.
[2] At the 0.05 level r = 0.11 and at the 0.01 level r = 0.15. These are approximations.

The data for the variability due to genetic causes present a different picture. The correlation coefficients are fluctuating about zero showing that probably very little of the genetic variances are accounted for by regression. In the studies of the genetic variances and covariances within and between segregating populations the degrees of freedom are sufficient to give a high degree of reliability. It will be remembered that for these data in populations A54-1 and A54-1BB in no case was more than 27 percent of

the genetic variability accounted for by regression (see Table 10). This was true for both the fertilized and non-fertilized plots. However for the segregating population 50-406BB on the fertilized plots the correlation coefficients were negative and as much as 69 percent of the variance of sucrose was accounted for by regression.

The corresponding environmental and genetic data involving the correlation coefficients and the percentage of the variances accounted for by regression are given in Table 13 for total nitrogen, betaine and glutamic acid. The associations are close and positive as regards both the environmental and the genetic components of variability. The exception is the genetic variability involving betaine and glutamic acid. Here only 27 percent of the genetic variance is accounted for by regression.

Table 13.—Correlation coefficients and percentages of the variances accounted for by regression, environmental and genetic.

Characters and variability	Correlation coefficient r	% accounted for by regression
Betaine vs. nitrogen		
Environmental[1]	0.88	76.7
Genetic[2]	0.72	51.9
Glutamic acid vs. nitrogen		
Environmental[1]	0.96	91.5
Genetic[2]	0.95	90.4
Betaine vs. glutamic acid		
Environmental[1]	0.84	70.4
Genetic[2]	0.52	27.2

[1] At the 0.05 level $r = 0.30$ and at the 0.01 level $r = 0.39$.
[2] At the 0.05 level $r = 0.11$ and at the 0.01 level $r = 0.15$. These are approximations.

Discussion

These researches show that much can be accomplished to improve the quality of sugar beets by cultural and fertilizer practices. Also they show that much can be accomplished by the plant breeder. However, to capitalize on the maximum potentials of the cultural and fertilizer practices and on the improved hybrids or strains produced by the plant breeder, the two must be combined into one effort. That is, proper cultural and fertilizer practices must be accompanied by the use of hybrids or varieties bred to give the desired performance under the cultural and fertilizer practices found to give the best results. To appreciate fully this statement let us again consider the data listed in Table 2.

From a comparison of the fertilized and non-fertilized plots of Table 2, it is clear that without exception the fertilizer used has increased the total nitrogen, betaine and glutamic acid in all six populations. These increases have been accompanied by significant decreases in percentage sucrose in three of the six popula-

tions but such is not true for the other three. Two of the three populations showing no significant decrease in percentage sucrose are hybrids and the other is inbred 52-307. The two hybrids have high percentage sucrose on both the fertilized and the non-fertilized plots, whereas, the inbred, comparatively speaking, has low percentage sucrose on both the fertilized and non-fertilized plots. It is clear that the two hybrids are capable of producing high sucrose at the higher fertility level.

Of considerable importance is the reason why the two hybrids are capable of producing high sucrose at the high fertility level, whereas, the commercial variety A54-1 and the broad genetic base A54-1BB derived from it are not capable of so doing. Consider further the data for A54-1 and the two hybrid populations 50-406BB and the F_1 (50-406 x 52-307) grown on the fertilized plots. The F_1 is lower in concentrations of total nitrogen, betaine and glutamic acid. These data indicate that the concentrations of nitrogen and the two nitrogenous compounds are rather closely associated with percentages of sucrose and hence probably of considerable importance.

This raises the question as to whether all genotypes react the same. The data are presented in Table 14. On the fertilized plots the data for A54-1 are 46.8, 116.6 and 80.1 and the percentage sucrose is 16.8, the data for 50-406 are 31.2, 125.3 and 17.3 and the percentage sucrose is 16.1, and finally the data for the topcross (50-406BB) involving this inbred are 33.6, 106.1 and 45.8 and the percentage sucrose is 17.3. These comparisons show that some genotypes have lower concentrations of betaine in the thin juice than others but do not differ greatly in the concentration of total nitrogen. Since 50-406 is high in betaine and 50-406BB is not, this might indicate betaine is more closely associated with low sucrose. However, this is not substantiated by the study of the environmental variances and covariances, as less of the environmental variance of percentage sucrose is accounted for by regression of sucrose on betaine than by sucrose on total nitrogen and sucrose on glutamic acid. The data as a whole seem to indicate that the two hybrids capable of producing high sucrose at the higher fertility level do so because the absorption, translocation and metabolic processes are such as to result in different concentrations of total nitrogen and total nitrogenous compounds in the thin juice as compared with the commercial variety and the inbred.

The data in Table 15 provide some information as to why the F_1 hybrid is capable of producing high percentage sucrose at the higher fertility level. Consider first percentage sucrose. The

Table 14.—Means and their standard errors for percentage sucrose, nitrogen, betaine and glutamic acid for A54-1, 50-406BB and 50-406.

Population and treatment	Sucrose	Nitrogen	Betaine	Glutamic acid
	%	mg	mg	mg
A54-1				
Fertilized	16.8±0.10	46.8±1.44	116.6±1.53	80.1±3.98
Non-fertilized	17.9±0.10	18.8±0.91	89.8±1.48	12.8±1.87
50-406BB				
Fertilized	17.3±0.10	33.6±1.32	106.1±1.33	45.8±3.50
Non-fertilized	17.6±0.09	12.6±0.62	69.9±1.40	6.4±0.93
50-406				
Fertilized	16.1±0.09	31.2±0.82	125.3±1.97	17.3±1.35
Non-fertilized	17.4±0.08	14.6±0.59	73.4±1.55	8.0±0.92

fact that 50-406 produced 17.4 percent sucrose on the non-fertilized plots shows that it has the potential for high sucrose production. That is, it has genes capable of conditioning high sucrose under the proper environment. On the other hand 52-307 does not have the genes for conditioning high sucrose but does have the genes that keep total nitrogen, betaine and glutamic acid low in the thin juice at time of harvest. The F_1 hybrid between 50-406 and 52-307 inherits genes for high sucrose production from 50-406 that are at least phenotypically dominant and genes from 52-307 that are phenotypically dominant for low total nitrogen and low betaine in the thin juice. Hence, the absorption, translocation and metabolic processes of the F_1 hybrid result in high percentage of sucrose at both fertility levels.

Table 15.—Means and their standard errors for percentage sucrose, nitrogen, betaine and glutamic acid for the two inbreds and their F_1 hybrid.

Population and treatment	Sucrose	Nitrogen	Betaine	Glutamic acid
	%	mg	mg	mg
50-406				
Fertilized	16.1±0.09	31.2±0.82	125.3±1.97	17.3±1.35
Non-fertilized	17.4±0.08	14.6±0.59	73.4±1.55	8.0±0.92
F_1				
Fertilized	17.6±0.09	21.3±0.58	101.3±1.20	18.0±1.18
Non-fertilized	17.6±0.07	9.8±0.31	51.3±1.14	3.5±0.28
52-307				
Fertilized	16.6±0.09	18.6±0.46	108.7±1.22	10.0±0.50
Non-fertilized	16.5±0.10	11.1±0.28	79.8±1.38	3.0±0.14

The data on the correlation coefficients and the percentage of the variances accounted for by regression show that there are interactions between genotypes and the environments. Further, the studies show that these interactions are such that it should be possible to make decided progress in improving the quality of the beets delivered to the factory through the joint efforts of chemists, soil scientists and plant breeders. Simply stated, sugar

beets can be bred which, if grown under specified and attainable cultural and fertilizer practices, will have the desired chemical constituents to meet the requirements for high processing quality.

Summary and Conclusions

(1) In this study there were three segregating and three non-segregating populations. They were grown at two soil fertility levels and there were 40 replications. The populations were randomized within treatments and replications.

(2) This design of the experiment provided a measure of environmental and genetic variability. There were three measures of the environmental variability: that due to differences between replications; that due to differences between plants in the non-segregating populations; and that due to differences between fertilizer treatments. There are two measures of the genetic variability: that due to differences between plants having different genotypes in the segregating populations; and that due to differences between means of the populations. The variances and covariances of the non-segregating populations furnish an estimate of the environmental variances and covariances of the segregating generations and hence provide a means of estimating the genetic variances and genetic covariances for these generations.

(3) In this experiment the differences between populations, soil fertility levels and replications for the characters percentage sucrose, total nitrogen, betaine and glutamic acid are statistically significant. This can be shown by tests (see Table 2). Such being the case these data should provide information on the interrelation of these characters. Variances, covariances, correlation coefficients and regression are calculated and used to study the interrelations of the characters.

(4) A high proportion of the environmental variance of percentage of sucrose was found to be negatively associated with total nitrogen, betaine, and glutamic acid. This showed that an increase in the applications of nitrogen-containing fertilizers resulted in an increase of total nitrogen, betaine and glutamic acid in the thin juice. Certain concentrations of these nitrogen constituents were accompanied by a decrease in percentage sucrose. The concentrations at which this decrease in percentage sucrose occurred were found to differ with populations. That is, there is a genotype environment interaction.

(5) The study of the genetic variability showed that the relations between percentage sucrose and total nitrogen, betaine and glutamic acid are much less marked. The genotype environment interaction was substantiated. This showed that genotypes differ in the amount of nitrogen constituents in the thin juice

and that this difference between genotypes is not necessarily the same for the two fertility levels.

(6) A study of the environmental variability for total nitrogen, betaine and glutamic acid revealed that all are positively associated and that the relation was closest for total nitrogen and glutamic acid. Glutamic acid and betaine were not nearly so closely associated. It seems that it would not be possible by altering cultural and fertilizer practices to vary total nitrogen much without affecting glutamic acid to some extent and in the same direction.

(7) A study of the genetic variability for the nitrogen constituents showed that total nitrogen, betaine and glutamic acid are positively associated and to nearly the same extent as found for the environmental variability. Again, betaine and glutamic acid are the least closely associated. Hence breeding programs designed to do so should be able to recombine these latter two nitrogenous compounds in different amounts. This finding may have considerable practical application to the breeding of populations of sugar beets for the production of both sugar and monosodium glutamate.

(8) The data indicate that decided increases in percentage sucrose can be obtained on high fertility soils by breeding populations of sugar beets adapted to growing under these conditions. In this study on an average such populations had lower concentrations of nitrogenous constituents in the thin juice. A study of the means showed that the two hybrids were such populations and were capable of producing high percentage sucrose at higher fertility levels.

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Effects of Beet Western and Beet Yellows Viruses on Amino Acids in Sugar Beet

JAMES E. DUFFUS AND J. M. FIFE[1]

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The beet western yellows (radish yellows) virus, an entity capable of inducing foliage yellowing on sugar beet indistinguishable from that induced by some isolates of the beet yellows virus, is prevalent in California beet-growing areas (1)[2]. This virus alone and in combination with the beet yellows virus can cause serious losses in sugar production and is considered the cause of a major sugar beet disease (2).

Changes in the concentration of certain amino acids take place in the leaves of sugar beet plants infected with the curly top virus (4) and also with the beet yellows virus (5). A ratio of the concentrations of certain of the amino acids which decrease to those that increase (aspartic acid plus glutamic acid/citrulline plus alanine) in plants infected with the beet yellows virus has been shown to have possible significance in selection of plants for resistance to the virus (5).

It was desirable to determine the effects of the western yellows virus alone and in combination with the beet yellows virus on the concentration of selected amino acids and especially the effects on the previously mentioned amino acid ratio. The results of these investigations are presented herein.

Methods

Leaf-tissue samples for amino-acid determinations were obtained from replicated field plots designed to determine the effects of the viruses on yield and sugar production. Plots 30 feet long and 3 rows wide were arranged in 4 Latin squares. The 4 treatments on the US 75 sugar beets consisted of uninoculated (control) plots, plots inoculated with a fairly virulent isolate of the western yellows virus, plots inoculated with a fairly virulent isolate of the beet yellows virus, and plots inoculated with both the western and beet yellows isolates.

One leaf disk, 3/4 inch in diameter, was removed from each of 12 mature leaves from different plants selected at random from each plot. The disks, taken approximately 2 months after inoculation of the plants, were composited for each plot and quick-frozen. The juice was expressed from the frozen disks at a pressure of 5,000 pounds, preserved in thymol and phenyl mercuric nitrate, and stored at 10° F. The samples were thawed,

[1] Plant Pathologist and Chemist, respectively, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Salinas, California.

[2] Numbers in parentheses refer to literature cited.

shaken thoroughly, and centrifuged before portions were removed for analysis.

Concentrations of the amino acids were determined by single-dimensional paper chromatography by the ascending method on 9 by 11 inch Whatman No. 3 mm papers, using a water-saturated phenol solvent. The techniques used were those described by Fife (5).

Results

The effects of the western and beet yellows viruses on the contents of certain amino acids are shown in Table 1.

Table 1.—Effects of the western yellows and beet yellows viruses on the concentrations of certain amino acids in sugar beet leaves at Salinas, California, 1959.

Virus used as inoculum	Aspartic acid (Mg %)	Glutamic acid (Mg %)	Citrulline + alanine (Mg %)	Ratio Aspartic acid + glutamic acid / Citrulline + Alanine
None (control)	13.64^{A}	24.06^{A}	23.96^{Aa}	1.607^{Aa}
Beet Yellows Virus	4.71^{B}	10.73^{B}	79.31^{Bc}	0.208^{Bbc}
Western Yellows Virus	4.71^{B}	11.36^{B}	59.96^{Bb}	0.291^{Bb}
Beet Yellows Virus + Western Yellows Virus	3.89^{B}	11.06^{B}	95.56^{Ba}	0.194^{Bc}

$^{A\,B}$ For a given trait, 2 means having any superscripts in common are not significantly different from each other at the 1% level.

$^{a\,b\,c}$ For a given trait, 2 means having any superscripts in common are not significantly different from each other at the 5% level.

Analysis of the aspartic acid and the glutamic acid data indicates a highly significant reduction in the concentration of these acids in the leaves of western yellows and beet yellows affected plants. The interaction of the effects of the viruses was also highly significant for both amino acids.

The factorial breakdown of the citrulline content analysis shows that the beet yellows virus affected the citrulline concentration much more than did the western yellows virus. The interaction was significant only at the 5 percent level. The Duncan comparison (3) shows that both yellows viruses increased citrulline significantly. The beet yellows virus and the combination of beet yellows and western yellows viruses significantly increased the citrulline content over the content for those beets infected with the western yellows virus only.

The analysis of the data for the amino acid ratio (aspartic acid plus glutamic acid/citrulline plus alanine) shows a highly significant reduction in the ratio in both beet yellows and western yellows affected plants. The interaction of the effects of the viruses was also highly significant. The ratio in beets infected with both

yellows viruses was significantly lower than in beets infected with the western yellows virus alone.

The western yellows and beet yellows viruses induced essentially the same qualitative effect on the amino acids studied in this experiment. The amino acid patterns induced by these 2 viruses were quite different from those induced by the curly-top virus (4).

Significant differences were noted between isolates of the western yellows and beet yellows viruses in relation to the concentration of citrulline in affected leaves. Whether reactions such as these may be used to distinguish between isolates of these viruses will be determined by further research using different isolates of the viruses and studies of their effects on additional amino acids.

It is noteworthy that the interaction of the viruses in regard to all the amino acids studied was significant.

The ratio of aspartic acid plus glutamic acid/citrulline plus alanine apparently is of little value in distinguishing beet yellows and western yellows viruses. If, however, this ratio proves to be of value in indicating resistance of beets to the beet yellows virus, it may also indicate resistance to the western yellows virus.

Summary

The concentrations of certain amino acids in leaves of sugar beet plants infected with the western yellows and beet yellows viruses were determined by paper chromatography. The concentrations of aspartic acid and glutamic acid decreased essentially to the same extent in plants infected with either the western yellows or beet yellows viruses. Citrulline plus alanine increased in plants infected by either virus. The beet yellows virus, however, affected the concentration of these amino acids to a greater extent than the western yellows virus. The interaction of the viruses in regard to all the amino acids studied was significant.

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CONSTITUTION AND BYLAWS OF THE AMERICAN SOCIETY OF SUGAR BEET TECHNOLOGISTS

(As Amended September 20, 1961)

ARTICLE I.

Name.

The name of this organization shall be the AMERICAN SOCIETY OF SUGAR BEET TECHNOLOGISTS.

ARTICLE II.

Objectives.

The object of this Society shall be to foster all phases of sugar beet and beet sugar research, and to act as a clearinghouse for the exchange of ideas resulting from such work.

ARTICLE III.

Membership.

Section 1. All persons, associations, corporations and other organizations interested in the advancement of knowledge concerning sugar beet production or beet sugar processing are eligible to membership in this Society.

Section 2. There shall be three classes of membership: (1) Individual membership, (2) Company membership, and (3) Honorary membership.

Individual members shall have the right to attend any meetings of the Society, to vote in elections and to secure one copy of such publications as are issued by the Society.

Company members shall be entitled to have one official representative attend any General Meeting of the Society, to cast one vote at elections, and to secure one copy of such publications as are issued by the Society.

Honorary members shall be entitled to the privileges of individual membership. Honorary membership is awarded as set forth in the Bylaws.

Section 3. Any member excepting an honorary member may be deprived of membership for nonpayment of dues or for other reasons by unanimous vote of the Executive Committee of this Society.

ARTICLE IV.

Sections.

In order to provide opportunity for the consideration of specialized subjects in branches of beet sugar and sugar beet research, sections of the organization shall be divided as follows:

A. Agronomy
B. Genetics and Variety Improvement
C. Entomology and Plant Pathology
D. Agricultural Engineering
E. Chemistry and Factory Operation
F. Physiology

Additional sections may be organized on a tentative basis for the purpose of facilitating presentation of papers with the approval of the Executive Committee. Upon evidence of ability to develop and execute a well-organized sectional program for two consecutive general sessions of the Society, sections

so organized may acquire permanent status upon approval by the Executive Committee and Board of Directors. A section may be terminated by (1) a two-thirds vote of the Society members in a mail ballot following 60 days' notice by the Executive Committee, or (2) if the section fails to present a program at each General Meeting, by a two-thirds vote of the Executive Committee and Board of Directors.

ARTICLE V.

Officers.

The officers of the Society shall consist of: President, Vice President, Immediate Past President and Secretary-Treasurer.

These officers shall constitute the Executive Committee.

ARTICLE VI.

Board of Directors.

The Board of Directors shall consist of seven members representing the geographical areas and technical interests as herewith listed:

Pacific Coast Region	1
Intermountain Region	1
Eastern Rocky Mountain Region	1
North Central and Great Lakes Region	1
Canada	1
Processing at Large	1
Agricultural at Large	1

Boundaries of these regions and technical interests will be determined by the Executive Committee and Board of Directors.

ARTICLE VII.

Election of Officers and Board of Directors.

The President shall appoint a nominating committee of five (5) members one year in advance of the General Meeting.

This Committee shall nominate two candidates for President, two candidates for Vice President, one candidate for Secretary-Treasurer and candidates for the Board of Directors, with a minimum of one more for each section than the number to be elected.

Ballots containing the nominations shall be submitted by mail to all members of the Society one month in advance of the General Meeting. Ballots may be returned by mail or deposited in the ballot box at the General Session up to 24 hours prior to the Business Session. Write-in nominations on ballots will be honored.

ARTICLE VIII.

Duties of the Officers.

Section 1. President. The President shall be the Executive Officer of the Society. He shall instruct the Secretary to call such meetings of the Executive Committee and the Board of Directors as he deems necessary. He shall preside over the General Meeting of the Society and meetings of the Executive Committee and Board of Directors. He shall, with the advice of other members of the Executive Committee, appoint the General Program Chairman, and such committees as may be necessary, and arrange for the special guest speakers who will participate at the General Meeting.

Section 2. Vice President. The Vice President shall serve in the absence of the President.

Section 3. Secretary-Treasurer. The Secretary-Treasurer shall keep the records of the Society. He shall attend to the collection of dues, keeping of accounts and payment of bills and obligations.

Section 4. Executive Committee. The Executive Committee shall act upon all matters within the jurisdiction of the Society which arise between the meetings of the Society and, in consultation with the Board of Directors shall formulate the policies of the Society as a whole.

ARTICLE IX.

Program Chairman.

The General Program Chairman shall, with the advice of the Executive Committee, appoint the chairman of each of the sections listed in Article IV, above. He shall be responsible for the preparation of the program for the General Meeting of the Society, instructing and advising Section Chairmen regarding their particular programs, advising the local arrangements committee regarding facilities and equipment necessary for the General Meeting.

Each section chairman shall develop and submit to the General Program Chairman a tentative program for his section, and shall be responsible for conducting the sectional meeting at the General Meeting of the Society.

ARTICLE X.

Time and Place of Meetings.

The General Meeting of the Society shall be held biennially. The place of the meeting shall be determined at the previous General Meeting of the Society and due notice shall be given to members.

Regional meetings may be held under the auspices of the Society upon approval by the Executive Committee.

ARTICLE XI.

Regional Organizations.

Regional meetings are recognized by the Society. The geographical and technical boundaries of these meetings will, from time to time, be described by the Executive Committee and Board of Dircetors.

The officers of each region shall consist of Chairman, Vice-Chairman and Secretary-Treasurer. If an organization does not exist in any of the regions, the President of the Society shall, if the Executive Committee approves of the regional meetings, appoint a Temporary Chairman, Vice-Chairman and Secretary-Treasurer, such officers to hold office until a regional meeting is held and regional officers are duly elected. These officers shall constitute the Executive Committee for their respective regions and shall be responsible for the appointment of necessary committees for carrying out the various projects proposed in their respective regions. All expenditures of funds by a regional organization shall be the responsibility of that organization.

ARTICLE XII.

Amendments.

Amendments may be proposed (1) by the Executive Committee directly, or (2) by petition of any ten members of the Society. Notice of the proposed amendments shall be submitted to the members at least 14 days before the ballot is taken. The amendment may be adopted by two thirds of the members present at any General Meeting.

If, in the opinion of the Executive Committee, the proposed amendment should be acted upon before the next General Meeting, it may be submitted to the members by mail. If it is approved by two thirds of those returning their ballots within thirty days, it shall be adopted.

BYLAWS

1. **Membership.** A candidate for admission to the Society shall make application on an approved form. Such form shall contain the candidate's interests in the Society as set forth in the Constitution. The completed form shall be submitted to the office of the Secreary who shall forward the information to the Executive Committee and Board of Directors. Upon unanimous approval, the candidate's membership shall become effective retroactive to the General Meeting immediately preceding his application.

2. **Dues.** Biennial dues for individual members shall be $5.00, and for company members $25.00 payable on or before the date of the General Meeting.

3. **Registration Fees.** A registration fee shall be charged those attending any General Meeting of the Society, both members and nonmembers. The amount of this fee shall be determined by the Local Arrangements Committee in charge of arrangements for the meeting and be subject to approval by the Executive Committee.

4. **Quorum.** A quorum for the General Meeting shall consist of 10 percent of the membership.

5. **Committees.** The President shall appoint such standing and special committees as seems desirable for carrying on the work of the Society. He shall appoint a Local Arrangements Committee, which shall be responsible for arranging for rooms for meetings, hotel accommodations for members and guests, the necessary equipment for conducting the meetings and entertainment for the biennial banquet of the Society.

6. **Publications.** All publications of the Society shall be under the direction of the Executive Committee and Board of Directors.

The publications will consist of a quarterly journal called "Journal of the American Society of Sugar Beet Technologists," and such other publications as the Executive Committee and Board of Directors may authorize.

The President shall appoint a Publications Committee consisting of five members.

The Publications Committee will have the responsibility of reviewing and passing on the suitability of papers to appear in Society publications. This committee will accept for consideration for publication manuscripts presented to the Society for that purpose. Papers presented at Society meetings have priority of publication. Acceptance for presentation does not assure acceptance for publication.

The Publications Committee will have jurisdiction over the release of papers and abstracts for use other than in the Journal. No commitments for the expenditure of the funds of the Society for publications can be made without the approval of the Executive Committee and Board of Directors.

The President, with the approval of the Executive Committee and the Board of Directors, will appoint one of the members of the Publications Committee as Publications Editor.

The Publications Committee will be guided by rules laid down by the Executive Committee and Board of Directors.

7. Awards.

Honorary Membership. An individual who has rendered outstanding service to the beet sugar industry, or has, by virtue of his scientific accomplishments, commanded the admiration and respect of this Society, may be nominated by the Board of Directors for Honorary Membership and elected by two-thirds vote by the Society at its General Meeting.

Honorary Membership includes all the privileges of a regular membership for life and without payment of dues.

Meritorious Service Award. This award for meritorious service shall be presented by the Society to members who have been outstanding in promoting the objectives of the society and have been active in the affairs of the Society.

Candidates for this award shall be nominated by an Awards Committee appointed by the President one year in advance of each General Meeting. Four months prior to each General Meeting, the Awards Committee shall present candidates' names to the Executive Committee and Board of Directors who will unanimously elect the recipients of such award. The Executive Committee and Board of Directors shall fix the number of awards to be presented during any one biennium, not to exceed 2 percent of the current membership.

Each awardee will be recognized with an appropriate certificate and citation on behalf of the Society at the General Meeting.

Forty-Year Veteran Award. Society members of at least four years' standing at the time of completing forty years of service to the beet sugar industry are considered as eligible candidates for the Forty-Year Veteran Award.

Candidates may be nominated either by their employer through the Society's Secretary or by the Awards Committee. After all nominations have been forwarded to the Board of Directors, unanimous approval is required for the candidate to be eligible to receive the award.

An appropriate certificate testifying to this award shall be presented to each recipient by the Society. Names of the awardees will be announced at the General Meeting and in a Society publication.